ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1958

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

14 Office of the Director

In the following pages are presented the highlights of accomplishment as seen by the Laboratory Chiefs and their responsible scientific and administrative staff. The NIAID is proud of the continued high quality of achievement catalogued in this report. The contributions to scientific knowledge and the positions of leadership assumed by the Institute as a whole, and by its various staff members individually, are clearly evident. Salient general items which should be given special mention are as follows:

1) Dr. Willard H. Wright, Chief of the Laboratory of Tropical Diseases, retired after an illustrious career as an internationally recognized authority on research in tropical medicine. His leadership will be sorely missed by the Institute. Administrative reorganization is presently planned which will make possible adjustments to insure that the research program in tropical diseases will be continued at its present high level and also make possible a continuation of activity through assignment of specific delegations and research missions to units of the present Laboratory.

2) In last year's report it was indicated that the Middle America Research Unit was being established. We are happy to report that this Laboratory is now a reality, with a full-fledged program and active research presently being pursued. This operation is strengthened through the joint participation with the NIAID of the Walter Reed Army Medical Center and through the happy collaborative activities with Gorgas Memorial Laboratory and the Panamanian Government, Ministry of Health.

3) The germ-free animal unit which was also noted as a beginning activity of this Institute in its last report now has an extremely active program under way and a very considerable background of experience in dealing with the technical difficulties of this system of research. Expansion of germ-free studies is anticipated and it is fully expected that new applications of this research system may be designed which will be of extreme value to the research programs of the Institute.

4) A Laboratory of Bacterial Diseases has been created during this reporting period. The research interest of this group will be directed primarily to physiological and genetic problems which surround the whole field of bacterial disease, especially fundamental research on resistant strains and mutations.

5) The extramural program of the Institute showed a wholesome growth during this reporting period. Especially noteworthy is the substantially increased support for research in staphylococcal disease and in cystic fibrosis, which was developed as a result of identified appropriations in these two areas. Probably the most fundamental contribution

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in the grants program, however, has been the opportunity to expand the training grants program to include the field of general microbiology as well as continue support in tropical medicine, virology, parasitology, and allergy and immunology. Also, the increase in the fellowship program has made possible a very substantial growth of our support of post-doctoral and special fellowships. During the previous years primary emphasis had been limited to the pre-doctoral fellowship program. During this year, with the increased support available, it was possible to extend fellowship awards to applicants in the advanced grades of post-doctoral and special. The expansion of this activity, together with the growth of the training grants program, will do much to help overcome the current shortage of trained microbiologists.

Estimated Obligations for FY 1959

Total:	$215,900
Direct:	131,800
Reimbursement:	84,100

Annual Report of Program Activities
National Institute of Allergy and Infectious Diseases
January-December 1958

LABORATORY RESEARCH

The three newly emphasized research activities mentioned in last year's report, allergy-immunology, tropical medicine, and germ-free animal studies have matured during 1958.

Research in immunology and the basic aspects of allergic reactions now occupies an active and established place in the direct research program of the Institute. A staff of ten scientists investigates the mechanism of immunologic and allergic reactions. In addition to contributing to the solution of basic questions in pathogenesis of disease, these studies will form a broad foundation for later clinical investigations. The Institute anticipates a beginning attack on clinical problems of allergic diseases employing the fundamental knowledge and techniques of scientists in the Laboratory of Immunology. Patient facilities of the Clinical Center allocated to this Institute will be available for these studies.

The establishment of the Middle America Research Unit, Panama Canal Zone in the fall of 1957, marked a significant advance in our efforts to re-emphasize research in tropical medicine. The Laboratory, co-sponsored by this Institute and the Walter Reed Army Institute of Research, now occupies suitable quarters near the Gorgas Hospital. Dr. Alexis Shelokov directs the investigations of four scientists and twenty technicians and assistants. Histoplasmosis is one subject of study. The greater effort, however, goes to studies of the arbor viruses and diseases associated with them. For instance, MARU scientists in the fall of 1958 examined the ecological and causative factors of an outbreak of eastern equine encephalitis among horses in the Pacora region of Panama. In this, they collaborated with scientists of the Gorgas Memorial Laboratory and of the Republic of Panama. The arbor virus section in Bethesda gave support by furnishing specific antigens and antisera and other special materials. This laboratory section in Bethesda developed concurrently with MARU and replaced some of the former activities in medical entomology.

Another disease of tropical areas receiving increased attention in this Institute is malaria. The work on malaria has progressed along the lines outlined in last year's report, namely, the development of a well rounded program in the pharmacodynamics of therapy. New knowledge is being applied to the chemotherapy and chemoprophylaxis of malaria and to inquiry into the exo-erythrocytic stage of the parasites, and attempts at tissue culture of plasmodial parasites.

The group working with germ-free animals occupied specially designed and constructed quarters during the year. This permitted accelerated work on experimental infections in guinea pigs and mice devoid of detectable bacterial life. Attempts at establishment of infections included those caused by amoeba, trypanosomes, trichomonads and helminths. A colony of germ-free white swiss mice was established and the second generation raised to maturity.

It has always been characteristic of microbiological and immunological research that its techniques are adaptable to the study of many supposedly noninfectious diseases. Knowledge derived from research in infectious diseases frequently has implications for all phases of medical and biological investigation. For example, NIAID virologists have turned their attention this year to the role of viruses in cancer. The researches of Gross, Stewart and Eddy have revealed a filterable agent cultivatable in tissue culture and associated with tumor formation in hamsters. Drs. Huebner, Rowe and others of the Laboratory of Infectious Diseases have applied the resources and specialized experience of their laboratory to characterization of this agent. They found it a highly resistant virus of a uniform antigenic constitution, as shown by serological and biological techniques. Frequently, it is a latent infection in laboratory mouse colonies. Their investigations have contributed to a clearer definition of its natural history and its relation to virus-induced cancers.

Increasing awareness of the importance of antibiotic resistant staphylococcus infections has stimulated inquiries into the mechanism of this resistance, its relation to pathogenicity and the factors responsible for virulence. Developing new information applicable to solution of the staphylococcus problem is a major objective of the newly formed Laboratory of Bacterial Diseases. This effort will also concentrate on other poorly understood aspects of bacterial diseases and on pathogenic bacteria such as Brucella, Pseudomonas, and bacteria characterized by production of reproductive filterable granules.

Of major importance to future work was a critical appraisal of current research activities in respect to administrative structure. As a result a new organizational plan evolved which divided research project responsibility among senior investigators more equitably, and reduced administrative tasks of the Chiefs of large laboratories. The new organizational plan has received administrative approval and is effective January 8, 1959.

Changes in clinical and research responsibility in the Laboratory of Clinical Investigation have delayed strengthening the cooperative clinical and laboratory approach to certain disease problems. The coordination of these two approaches is expected to bring greater progress during the coming two years.

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A definite trend now has become apparent toward an interest and stress on research projects of a fundamental nature such as biology of viruses, cell nutrition, and antigenicity of cell components. Ecology, epidemiological and therapeutic studies continued to maintain their previous high quality of design and critical analysis but occupied a relatively smaller proportion of total Institute effort. The activities pursued at the Rocky Mountain Laboratory illustrates the dual approaches to unsolved problems of human disease.

COLORADO TICK FEVER

Investigations of Colorado tick fever have continued and have given valuable information on the ecology of the disease as well as on the clinical, immunologic, and diagnostic aspects. Studies performed in Montana and Colorado show that many of the smaller mammals which are hosts to the various stages of D. andersoni may be infected with virus and maintain a long period of viremia. Among the mammals infected in nature are the golden-mantled ground squirrel, Columbian ground squirrel, porcupines, deer mice, and chipmunks. The intimate relations among ticks, mammals, and virus are indeed complex, as the incidence of infection in arthropods and mammals varies from one biotic area to another even though these areas may be practically contiguous. The degree of infection in ticks appears to be dependent upon the number of animals displaying viremia, which again is dependent upon their previous experience with the virus. Since we have accumulated overwhelming evidence that virus is not transmitted transovarially by ticks, the effect of the virus on mammalian hosts becomes of paramount importance for determination of the reservoir of the disease and control and prevention of infection in man.

Attempts to immunize man have been impeded for a number of reasons. Foremost among these has been the instability of the antigen contained in vaccine. Although freshly prepared vaccine was shown to protect mice and to be innocuous for man, it was not possible to give booster doses of vaccine to human volunteers because the vaccine had lost its potency. Other lots of vaccine are being prepared by the same and by other techniques than were employed to manufacture the first preparation. These are being tested and will soon be applied to human subjects to determine if a booster dose will produce measurable neutralizing antibodies.

Successful development and application of tissue culture techniques to the isolation and identification of CTF virus and to the titration of neutralizing antibodies against the virus have provided tools for diagnosis which can be employed by State and local health laboratories. These methods, coupled with the complement-fixation test which has been developed, should enable us to free our facilities of much of the diagnostic work we have done in the past.

TICK PARALYSIS — Tick paralysis for many years has been known to occur in man and animals during the period when female ticks were completing engorgement. Only in the last two or three years has there been any real progress in this field. Following the observation that marmots developed paralysis when ticks fed on them, Hughes and Philip demonstrated that hamsters were susceptible to the disease. After female ticks have fed for six to seven days, the hamsters become paralyzed. If the ticks are not removed, the animals die in a few hours and at autopsy the most prominent and consistent findings are congestion of the lungs and accumulation of moderate to large amounts of fluid in the pleural cavities. If the ticks are removed shortly after paralysis is definite, the animals recover. Recovered animals show no evidence of immunity. Pharmacological studies indicate that the agent acts primarily at the level of the central nervous system and secondarily acts at a peripheral level.

After a female tick has caused paralysis in an animal, the tick may be removed and placed on a series of animals in which paralysis will be produced as frequently as it will attach and feed. In a single test we have found that the agent persists in the F1 generation of ticks established from a single female tick obtained from a girl suffering from tick paralysis.

BAT RABIES — It is of some interest to note that at the same time a fatal case of rabies in a woman bitten by a bat was reported by the California State Board of Health it was demonstrated for the first time at the Rocky Mountain Laboratory that a naturally infected bat can produce rabies in mice by biting. This is the first known instance of transmission of rabies by bats. It is likewise significant that treatment with vaccine and serum failed to influence the outcome of the disease in the human case. Studies in progress in Hamilton are directed toward the question of immunity and the development of resistance to infection by suitable procedures.

WESTERN EQUINE ENCEPHALITIS — As is well known, progress in the study of arbor viruses has been extremely slow, probably because of the paucity of fresh ideas which might be used to examine the ecology of the diseases in question. It is becoming increasingly evident that the known ecological facts will be explicable only on the basis of resident species of mosquitoes and vertebrate hosts. The idea that mosquitoes might be the means by which the WEE virus survives the winter in the northern United States was supported by our finding mosquitoes which actually over-wintered in rock piles in eastern Washington. When it was published by others that only females that

had taken a carbohydrate meal developed fat-bodies necessary for hibernation, the original idea no longer appeared tenable. It was found, however, that females which have taken a blood meal will subsequently take a carbohydrate meal and develop fat-bodies. It is, therefore, still possible that infected mosquitoes might be the source from which each year's supply of virus is replenished.

On the other hand, some non-migratory vertebrate could be the storehouse for virus. The observation that garter snakes can be infected with WEE and EEE virus and that they display viremia at high titers for considerable periods of time suggests that cold-blooded animals should be examined to determine the various factors influencing experimental infections and to determine whether or not infected or recovered specimens can be found in nature. To date, no evidence has been accumulated to show that snakes are naturally infected, but larger numbers of specimens collected during several seasons must be examined before it can be stated whether or not snakes play a role in the ecology of the arbor viruses.

RICKETTSIAL INFECTIONS — During the last year or so, interest in rickettsial diseases has been revived, and considerable research has been done. Probably the most valuable laboratory observation has been that rickettsial suspensions and soluble antigens derived from rickettsiae may be purified by chromatography. The obvious application of this technique to studies of the enzyme systems and metabolic pathways utilized by these organisms is being investigated and, on the basis of this type of research, it may be possible to determine whether or not rickettsiae will grow on cell-free media. In addition to these studies, considerable effort is being made to classify rickettsia belonging to the spotted fever group. In general, the results of tests done with complement-fixation reactions with soluble antigens and the mouse neutralization test are in agreement. Siberian tick typhus rickettsiae, although they can be differentiated from other rickettsiae in Type A1 (R. rickettsii) by the latter test, appear to belong to this group by the former reaction. This is the first time that R. rickettsii has been isolated from the Eastern Hemisphere, although the epidemiology would place it in the A2 group.

Q FEVER — Q fever has recently assumed a position of high importance in our program. The discovery of 87 human cases in Idaho last year prompted studies of the animal reservoir in the area. While the incidence of infected herds was in the neighborhood of 1% in 1951, it had increased to roughly 15% by 1958. Incidental to these findings, surveys were made in Montana, and a number of infected herds were discovered. Some of these herds are in the Bitterroot Valley, an area which we had previously studied with negative results. Surveys conducted in cooperation with many State and local health groups have demonstrated the widespread occurrence of Q fever in man and animals in North America.

SALMONELLA ANTIGENS — Studies of immunity against Salmonella spp. have progressed rapidly. It has been shown that Vi antigen can be obtained in a purified state and that two or more chemically distinct types exist. Hydrolysis of Vi antigen results in partial degradation with resultant loss of certain types of biological activity, including protection of mice against challenge with S. typhosa. Contrary to the findings of many investigators who have found the activity of "complete antigen" or endotoxin of Gram-negative organisms to be related to the lipid content, RML scientists have shown that it is possible to remove lipids leaving behind a carbohydrate-protein complex which retains all the activity of the "complete antigen." A mouse-neutralization test has been devised. This is a test for antibacterial rather than antitoxic immunity and therefore fills a great need in the methods and techniques for evaluation of immunity against salmonella infections. It is a valuable method of assay of antigens since it is specific, reproducible, and displays a dose-response curve. In addition, a simple method for evaluation of activity of endotoxin has been described. This method, which requires very little material, is a very simple one which employs intracutaneous injection of rabbits with measured doses of material. The results are highly reproducible and check closely with those obtained in standard mouse tests.

There have been significant advances in our studies of tuberculosis. At the outset the susceptibility of mice to pulmonary infection was determined and it was found that a uniform disease picture could be produced. Original tests to determine if mice could be immunized with living suspensions of B.C.G. or H37Ra yielded excellent results. Subsequent experiments were, however, disappointing. These experiences appeared to be due to use of organisms for immunization which had been subcultured for a number of times on Dubos liquid medium. An experiment was set up to determine if organisms lost their immunizing potency after continuous passage on this medium. Two strains of B.C.G. and one of H37Ra were passed 2, 4, and 9 times in Dubos medium and used to immunize mice. It was found that mice immunized with organisms passed two or four times in liquid medium were immune, whereas those immunized with organisms passed 9 times were relatively susceptible to infection with virulent tubercle bacilli. Since all studies on the immunogenicity of cell walls and protoplasm had been performed with organisms passed many times on liquid medium, it is no wonder that they yielded uniformly negative results.

ANTIGEN FRACTIONS — Recently, a system has been developed whereby large amounts of cell walls can be harvested from the bacilli by rupturing the organisms under pressure. By this method as much as 50 milligrams of cell walls have been obtained in a single run.

Cell walls of M. butyricum have been treated in a Raytheon ultrasonic apparatus and the resultant suspension centrifuged at high speed. The supernatant fluid contained fine, translucent material and minute granules and the sediment contained large granules similar to those described by Youmans. These fractions, together with cell walls, were injected into the skin of normal rabbits. The sonic sediment failed to produce notable lesions, while the cell walls produced typical lesions. The sonic supernatant produced intense lesions to a higher titer than did the cell walls.

Since our conception of immunity in tuberculosis is based on a nonviable antigen and depends on material which will not cause delayed hypersensitivity, this initial fractionation of cell walls into two distinct portions (based on activity in normal rabbits) suggests that an immunizing antigen can be obtained by the present approach.

In addition to the previously mentioned studies of cell walls, considerable progress has been made in similar investigations of other organisms. Heat-labile toxins of Pasteurella pestis and Hemophilus pertussis, which are lethal for mice, have been shown to be protoplasmic constituents. Cell walls of P. pestis contain a heat-stable fraction which produces dermal lesions in normal rabbits. The results suggest that exotoxins are integral constituents of the cell and are not secreted into media but are released following lysis of bacterial cells. In electron microscope pictures of P. pestis subjected to continued washing, the release of protoplasm is neatly demonstrated.

Isolation of a purified fraction consisting of a protein-carbohydrate complex from Histoplasma capsulatum has been accomplished. This fraction has been characterized chemically and physically. While it does not fix complement in the presence of immune serum, it is capable of stimulating immunity in mice against fatal infections with H. capsulatum and may be employed for skin tests for the diagnosis of histoplasmosis. In the latter use, the fraction overcomes many of the objectionable features of histoplasmin, as the degree of specificity is marked. The protein portion of the material appears to be responsible for the hypersensitivity reaction. Antigens have also been isolated from Trichophyton rubrum and these are being used in human subjects to determine their effect on dermatomycosis in man.

EXPERIMENTAL HYPERSENSITIVITY — The program relating to allergy has as its objectives an understanding of the mechanisms of hypersensitivity and the relations existing between delayed hypersensitivity, on the one hand, and immediate hypersensitivity and the presence of circulating antibody, on the other hand. It has been established that there is a temporal relation between these types of classical immunity. Following injection of such materials as diphtheria toxoid or crystalline egg albumin, guinea pigs develop delayed hypersensitivity, which persists from about the fifth to the eleventh day, whereupon immediate hypersensitivity associated with demonstrable

antibodies becomes manifest. Irradiation of guinea pigs with 200 to 300r of X-irradiation 18 to 24 hours prior to sensitization does not influence the development of delayed hypersensitivity, but the occurrence of circulating antibodies is delayed until about the 21st day. If the dose of X-rays is decreased, the development of immediate hypersensitivity is again delayed in proportion to the size of the dose of irradiation. If X-rays are withheld until 12 to 24 hours after administration of antigen, no influence is noted upon the occurrence of either type of hypersensitivity. These results show that manifestation of delayed or immediate type of hypersensitivity following injection of purified antigens into animals is primarily due to the host responses and that separate mechanisms must be responsible for production of the types of hypersensitivity since they may be influenced so differently by administration of X-rays prior to but not after administration of antigen.

RELATIONS OF VIRUS TO HOST CELLS

The advances being made in the study of the relations of viruses and host cells are of great interest and importance. While the ease with which virus may be purified is astounding, this step is considered only a technique which is necessary for the ultimate objectives of the program. The primary aim of the program is to determine the steps required for the production of new virus in mammalian cells and to obtain protein fractions which are antigenic but nonviable. The method of purifying virus has been described previously with electron microscopic photographs of purified virus. Recent photographs show the virus to be arranged in a crystal-like fashion with particles arranged at 60° or 90° angles and each particle appears to have a central core which is probably nucleic acid. The purified virus agrees in all respects (chemically, physically, and biologically) with the preparations made by Schwerdt and Schaffer. By raising the pH of these preparations with ammonia vapor, the virus particles disintegrate into rodlike elements and small particles. The nature of the rodlike elements is not clear, but most certainly they constitute rearrangement of one or both of the original portions of the virus particle.

The virus may be dissociated with urea. With 5.2M urea the virus is partially dissociated, but with 8M urea its components are completely separated. The protein portion is immediately eluted from DEAE columns by 0.05M phosphate buffer in 5.2M urea, but the nucleic acid is eluted only by increasing the salt concentration and lowering the pH. The protein fraction contains 1% of the P^{32} and the balance of the P^{32} is contained in the nucleic acid portion. Since the protein is eluted in the same way as virus, it is evident that the column behavior of poliovirus is determined by its protein coat. While protein

obtained by dissociation with 8M urea is not antigenic, presumably due to denaturation, protein obtained with 5.2M urea and still containing 10^3 infectious units is antigenic. Ordinary virus preparations containing 10^3 infectious units are not antigenic, so it is evident that the dissociated material has antigenic components in excess of the viable virus.

Virus protein may be separated from normal cell protein by elution on TEAE columns and by differential centrifugation of eluates from DEAE columns.

RESPIRATORY DISEASES — Respiratory disease and common cold research at Bethesda led to the definition of a new group of viruses, the "parainfluenza" viruses, including the discovery of two new agents, HA-1 and HA-2.

The new parainfluenza viruses were shown to represent universal infections of the respiratory systems of man, having unique and frequent abilities to produce infections and illness more than once in the same persons.

In collaboration with Children's Hospital Research Foundation of D.C., and the D.C. Welfare Department, the extensive Laboratory of Infectious Diseases field studies of respiratory illnesses in young infants and children showed that these agents caused more illness than Asian influenza and adenoviruses -- the illnesses ranging from mild febrile respiratory illnesses to croup and pneumonia.

Parainfluenza viruses, seldom demonstrable without special techniques, were also found in monkeys, guinea pigs, and in various tissue cultures, including monkey kidney tissues used for viral diagnostic studies and poliovirus vaccine. Their importance in these interesting hosts and circumstances is under study but still remains to be determined.

Technical advances greatly facilitated the identification and characterization of thousands of newly recognized viral strains isolated from the LID-wide studies of the microbial experiences of infants and children. These included: 1) Several simple and standardized methods for using the hemadsorption technique (discovered in this Laboratory in 1957) in large scale studies of parainfluenza infections in man and cattle; 2) The discovery of hemagglutinins in adenoviruses for red blood cells of various animals, and a hemagglutination-inhibition test which greatly simplifies specific identification of adenoviruses and specific diagnoses of adenovirus infections; 3) Development of accurate and simple complement-fixation techniques for identifying more than 50 enteroviruses, including Coxsackie, ECHO, and polioviruses, many of which contribute to their prevalence in common, undifferentiated respiratory diseases, especially in children. These techniques save large amounts of time and money compared to other techniques available and also are of value in serologic epidemiologic studies of the prevalence of the various diseases caused by these viruses.

The importance of respiratory disease research is indicated by the fact that more than 40 percent of the respiratory illnesses observed in our pediatric study populations (Children's Hospital and Jr. Village; nearly 3000 children observed) were explained during 1958 by the following viruses - influenza (Asian), Parainfluenzas (HA-1, 2, and CA) and by adenoviruses. If this 40 percent figure (probably a minimum one), which will be augmented when the contributions of numerous other virus infections observed (enteroviruses, etc.) are taken into account, can be taken as representative of childhood experience in general, then millions of unclassified respiratory illnesses can now be explained and perhaps prevented.

RESPIRATORY DISEASES IN CATTLE — In collaboration with Animal Research Services of USDA, HA-1 or parainfluenza 3 was also shown to occur extensively in cattle. Preliminary studies of outbreaks and surveys of widespread infections in the East and Mid-west show a relationship of this virus to bovine respiratory diseases and to shipping fever -- both serious economic problems. Possible spread of the virus from cattle to man (or vice-versa) must be considered, but has not yet been studied.

VACCINE STUDIES — A number of experimental killed-virus vaccines were produced and tested in experimental animals volunteers, and, for the first time, also in children. These include measles, polyvalent adenovirus, parainfluenza, and Coxsackie vaccines The vaccines produced no recognizable deleterious effects. Potency tests indicated that all of them have antigenic value as measured by quantitative immunological procedures. Their abilities to prevent human disease, particularly in children, were tested in small scale field trials. Depending on the results of these trials, their actual value must be measured by larger-scale field trials.

Adenovirus vaccine studies in military recruits (with the U. S. Navy Medical Research Unit #4 at Great Lakes Naval Training Center) were completed. Final analysis revealed excellent protection against acute respiratory diseases, significant reduction in hospitalizations, and a remarkable reduction in the incidence of pneumonia.

Vaccines prepared against the human and cattle strains of parainfluenza showed excellent potency in immunity tests in guinea pigs, rabbits, and calves. Their possible value in preventing human and bovine disease was placed under study late in 1958.

CANCER VIRUS STUDIES — Precise studies of the natural history of a tumor virus in its natural host were made possible for the first time by the development and application of new and standare virological techniques to the mouse polyoma virus.

Information on this most interesting virus, grown in tissue culture and shown to cause 41 cancers in mice, plus eleven in hamsters by Stewart and Eddy, of NCI and USS respectively, was further developed.

Polyoma virus was shown: 1) to be immunologically different from 88 known viruses of murine and human origin. With Dr. Kahler of NCI, its virus nature was first visualized and its size determined; 2) to be excreted in enormous amounts in saliva, urine, and feces of infected mice; 3) to spread readily amongst mice by both respiratory and intestinal routes - resembling in this respect the spread of common respiratory and enterovirus diseases; 4) to occur as a contaminent of numerous transplantable tumors used in cancer chemotherapy and as frequent fellow travelers in Gross leukemia and Schwartz leukemias.

Mouse polyoma virus was found to resemble many of the common non-tumor viruses, not only in its properties, but in its behavior as an infectious agent. Nearly all of its naturally occurring activity in the mouse and in mouse populations is inapparent; clinical manifestations (in this case, cancer) are extremely rare even when natural infection is nearly universal. As is also the case with many non-tumor virus infections, understanding and eventual control of a tumor virus will depend on eventually obtaining full information concerning the whole process of the virus infection. The ability to precisely determine the large submerged part of the tumor viral iceberg, is the most important development in tumor virus research, and this was achieved, as is always the case in any viral research, through the development of quantitatively precise techniques or tools for measuring virus growth and spread, independent of the production of tumors. These include standardized and carefully evaluated hemagglutination-inhibition and complement-fixation tests for antibody determination, and a simplified, very sensitive antibody production test for large scale studies of virus activity in tissues and excretions. It should be stated that this rapid progress was in large part made possible by Gross and Stewart, who discovered the virus, and Stewart and Eddy, who first grew and characterized the virus in tissue cultures.

Recently, LID virologists developed a complement fixation test for another mouse tumor virus -- Friend leukemia. This should permit surveys to define the true natural history of this virus as well.

CELL BIOLOGY It has become increasingly apparent that dispersed cell cultures constitute a remarkably flexible and useful tool for the study of mammalian physiology at the cellular level. Institute scientists have embarked on a broad study of the nutritional requirements and metabolic activities of cell cultures deriving from a variety of tissues, both human and animal, normal and malignant, adult and embryonic. Carbohydrate metabolism, amino acid biosynthesis and interconversion, protein synthesis and turnover, and nucleic acid biosynthesis are some of the specific projects in progress. In the course

of the past few months it has further become evident that mutants arise in these cultures which can be selected in an appropriate environment; and a number of markers have been identified. Studies on genetic variation and recombination have thus become feasible with animal cells in dispersed cultures, the full implications of which can only be guessed.

The interaction of viruses with cultured cells is also being investigated, with specific reference to 1) the nutritional requirements for poliovirus synthesis, 2) the isolation and chemical characterization of poliovirus, 3) an analysis of the carrier state and interference, and 4) the early effects of viral infection on cellular metabolism.

BIOLOGY OF VIRUSES — This work has had one central theme concerning the principles and mechanisms involved in virus attachment, penetration, multiplication and release from infected cells. Most of the effort has been aimed at the cellular level but several projects have considered these problems in the intact animal, thus introducing immunological factors not present in tissue culture systems.

In contrast to most current studies of virus-host relationships, attention has been focused more on the very early stages of infection before the appearance of new intracellular virus. Since one of the practical goals of this basic work is chemotherapy of virus diseases, it seemed more logical to think of an effective metabolic block being found for the early phenomenon of attachment, penetration, and establishment of templates for synthesis of viral protein and nucleic acids. Both biological investigations in tissue culture systems and biochemical in infected animals have already indicated the importance of cell membrane and perinuclear microsomes in these early stages of infection.

AUTOLYSIS IN GENUS BACILLUS AND RADIATION SICKNESS — A project in collaboration with Dr. Eugene Weinberg, Department of Bacteriology, Indiana University, has indicated that cysteamine and various derivatives of it (AET, AFT) are capable of inducing lysis within six hours of post logarithmic cells of strains of Bacillus subtilis. An interesting correlation has been observed. Thus far, cysteamine derivatives which have the capacity to protect against radiation sickness are also able to induce lysis. In an attempt to learn something of the mechanism of the induction of lysis by these compounds, it was found that the cysteamine derivatives were capable of sharply inhibiting the auto-proteolytic activity of extracts of sensitive organisms. As above, there has been correlation between the ability to inhibit auto-proteolysis and the ability of these compounds to protect against radiation sickness. Compounds

differing only slightly in chemical structure from AET, AFT and cysteamine do not lyse the organisms and in addition have been reported to be without effect on radiation sickness.

There is at the moment no explanation for the apparent correlation between lysis of members of the genus Bacillus and protection against radiation sickness. A complicating factor in this investigation has been the extreme variability in culture of members of the genus Bacillus with a concomitant variation in susceptibility to the induction of lysis and inhibition of proteolysis. Nevertheless one potential use of the lytic phenomenon may be its use as a screening agent for radiation-sickness prevention compounds. It takes but six hours to run a lysis experiment and up to 28 days to determine the effectiveness of a potential drug for use vs. radiation sickness in mice.

DRUG DETOXIFICATION — A complicating factor in the widespread use of the tuberculostatic agents, streptomycin, isoniazid and cycloserine, administered separately or given in combination, is toxicity. Toxicities have been reduced to the extent that three to six times lethal doses could be given to mice by simultaneous injection of various adjuvants such as certain amino acids, certain vitamins, glycerine, aromatic sulfonic acids, etc. These decreased toxicities do not result in any loss in in vitro bactericidal action of the chemotherapeutic agents. Further, detoxification of certain toxic parasiticidal agents such as chloraquine, daraprim, pamaquine, pentaquine, isopentaquine, primaquine, Miracil D, Hetrazan and Hoechst 698, and fungicidal agents, mycostatin and ascosin, has been achieved by treatment of these agents with various aromatic sulfonic acids. As in the case of the tuberculostatic compounds, again, there was no interference of the chemotherapeutic activity of the drugs in vitro.

Since all of the above-mentioned adjuvants have low toxicity in man, the results indicate their possible utilization to increase tolerance to widely used but more or less toxic drugs. Obviously, greater therapeutic efficiency against infections may be achieved in this manner and further, massive doses of the detoxified chemotherapeutic agent may result in decreased rates of emergence of resistant strains.

MEDICAL MYCOLOGY — Studies are oriented about the role of fungi in human disease. Continued investigations of their distribution in nature confirm the saprophytic occurrence of Coccidioides in areas sheltering pigeons, and reveal an association of Histoplasma and the house bat.

Because of the continuing need for efficient anti-fungal agents, studies of various drugs against Histoplasma, Coccidioides, Blastomyces, and Cryptococcus are still being vigorously pursued. New chemicals are being tested in animals, and if warranted, may be given clinical trial in collaboration with investigators of LCI. Collaborative studies are also being conducted in methods of detoxification. One effective new agent has been found, and others are promising.

Of obvious importance in this project is basic knowledge of the metabolism and physiology of pathogenic fungi, and changes induced by anti-fungal agents. Improved culture methods for Coccidioides have been devised to allow future metabolic studies. The tolerance of Candida albicans to Amphotericin B has been shown capable of increase in vitro, and alteration of some of its basic properties under these conditions has been observed.

Immunity in fungal infections is of importance, but little understood. Studies of the immunizing capacities of several fungi, particularly with respect to protection against active infection, are underway; and antigenic analysis of others by adsorption of the immune sera produced is also in progress. Of related interest is the problem of virulence of fungi: with the organism tested (C.albicans), virulence has been shown to vary considerably among strains.

BRUCELLOSIS — Long term studies on brucellosis at Bethesda and in collaboration with Purdue University have continued to clarify many of the basic and practical problems concerning this disease. For instance, when swine are experimentally infected with Brucella melitensis they exhibit the same generalized and prolonged disease as that produced by Brucella suis. A recently described species, Brucella neotomae induced infection of moderate degree in swine which was also similar in clinical characteristics.

MALARIA — One of the questions incompletely answered is the role of excerythrocytic stages in the pathogenesis of malaria. Institute malariologists are exploring these questions with Plasmodium cynomolgi in monkeys. They can induce the previously described characteristic bodies in the liver by injection of infected salivary glands from eight or more infected mosquitoes. There is a positive correlation between the number of bodies in the monkey liver, the number of infected glands injected and the resulting parasitemia. The bodies first appear four days after inoculation of sporozoites, enlarge in size through the ninth day, then diminish in number with the advent of parasites in the blood. Primaquine and pyrimethamine treatment clears the liver of the bodies. However, pyrimethamine treated monkeys develop malaria forty days later, indicating survival of some of the tissue-inhabiting parasites.

The pharmacologic action of antimalarials has been explored by a number of approaches. For instance, after injection parentally in the rabbit, triturated pyrimethamine is excreted slowly and two metabolites appear in the urine. Oral administration of the free base of pyrimethamine to the mouse failed to yield reproducible levels. Preliminary results suggest that the antimalarial activity of such compounds as chloroquine may be prolonged when administered in conjunction with an inhibitor to the metabolism of the drug.

PYRIMETHAMINE RESISTANCE BY PLASMODIA — Of concern to malariologists is the tendency of strains of human plasmodia to develop resistance to pyrimethamine. Clinical studies at the South Carolina State Hospital, showed that resistance by P. falciparum developed rapidly after an initial dose of .25 mgm. Both schizontocidal and sporontocidal effects were lost. Increased drug dosage did not overcome the resistance. The factor was transmitted apparently undiminished through the mosquito cycle. Similarly, resistance by P.vivax developed after initial doses of either 25 or 100 mg. of pyrimethamine and persisted through transmission by mosquito bites, sporozoite injection, or blood transfusion.

Primaquine in small doses, evoked an early sporontocidal action against P. vivax and P. falciparum. This may lead to practical use in malaria eradication efforts.

HOOKWORM THERAPY — Extensive trials of a new drug, bephenium, have been done, representing the first major controlled investigation of the drug against human hookworm. The more effective regimens have produced up to 85% reduction in worm burden. Trials with the new anthelmintic drug, dithiazanine iodide, have confirmed the effectiveness of this drug against Trichuris.

Partial development of the parasitic cycle of the human hookworm, Necator americanus, has been obtained in vitro. The free-living stages were successfully reared in previous work, so that much of the life history of this parasite can now be studied in vitro under xenic conditions.

Using improved media for rearing the free-living and parasitic stages of Nippostrongylus muris, considerably higher yields of adult worms have been obtained. Females have deposited large numbers of eggs in culture, but these eggs have all been infertile.

The investigation of the nitrogenous excretory products of tapeworm, T. taeniaformis, larvae has shown the presence of ammonia, urea, aliphatic amines, amino acids, and a small amount of polypeptides.

These findings are similar to those previously obtained from certain round worm larvae except for the presence of urea which was shown to be absent in the case of the round worms.

The preliminary report that the chromosome number of Diphyllobothrium ursi is N=9, 2N=18, was confirmed. Four of the paired chromosomes are very small.

EXPERIMENTAL SCHISTOSOMIASIS — A syndrome that closely resembles hepato-splenic schistosomiasis in humans was reproduced in mice. It was determined that the pathogenesis of the syndrome was related to the penetration of the portal canals by large numbers of schistosome eggs causing obstruction of portal blood flow. No specific alteration in parenchymal liver cell function was detected.

PARASITE PHYSIOLOGY — Studies on metabolism and chemical composition of parasites contribute to understanding of the pathogenesis of parasitic disease and may provide a rational approach to chemotherapy. Biochemical studies on the effect of pentachlorophenol on muscle metabolism for instance, showed that it exerts a dual action upon myosin ATPase similar to its effect upon mitochondrial ATPase; low concentrations enhance phosphatase activity and high concentrations inhibit it. A previous study showed that oxidative phosphorylation was impaired in the liver and brain of aged rats. Subsequent work reveals lowered contents of pyridine and adenine nucleotides in brain and liver mitochondria of the aged rat, which may be related to the earlier observation.

TOXOPLASMOSIS — Studies of Toxoplasma cysts have shown that the cyst wall is immediately digested by pepsin or trypsin; the liberated parasites can survive briefly in pepsin and longer in trypsin. Cysts are rendered non-infective by brief heating or immediately on being placed in distilled water.

Congenital transmission of toxoplasmosis from chronically infected mother rats to their offspring has been found to occur only rarely. It occurs more frequently in mice infected with a cyst-producing strain of Toxoplasma. Residual infection in the uterus has been demonstrated in chronically infected rats and mice, suggesting a means for congenital transmission without a generalized exacerbation in the mother.

In a study of residual infection in human uteri, Toxoplasma cysts were demonstrated in the uterus of a woman with serological evidence of past infection. Toxoplasma has been demonstrated in [illegible] of [illegible] samples of skeletal muscle from sheep slaughtered in Baltimore.

Detailed information has been gathered on the stability of the hemagglutination test antigen in the liquid and solid states at various temperatures. Powdered antigens are stable at high temperatures. In acute toxoplasmosis, the hemagglutination test reveals antibodies about four days later than the dye test; there is evidence that this may be due to an inhibiting substance in the acute serum, rather than to different antibodies. Eventually hemagglutinating titers reach the same levels as those found by the dye test. Hemagglutination-inhibiting substances are found in some small animal sera.

Bio-assays of pyrimethamine in the serum of rabbits following one dose of 100 mg/kg showed decreasing levels up to 7 hours. Pyrimethamine was detectible in the retina of a monkey. Also, in guinea pigs treated with pyrimethamine and sulfa following inoculation of Toxoplasma into the posterior chamber of the eye, chorioretinitis did not develop, and parasites were only rarely recoverable from the ocular tissues.

HISTOPLASMOSIS IN PANAMA

Since the initiation of investigations in May 1958, the existence of acute histoplasmosis on the Isthmus of Panama has definitely been established. In cooperation with the Isthmian medical facilities over thirty cases of histoplasmosis have been serologically confirmed. Attempts to recover H. capsulatum from acute cases of histoplasmosis have been unsuccessful as was to be expected from the experience of previous investigators. The animal studies indicate that spiny rats, which comprise 80 per cent of trapped ground mammals in Panama, can be infected with H. capsulatum and are undoubtedly infected in nature. Spiny rats collected locally have been found to possess complement-fixing antibodies and skin hypersensitivity to histoplasmin. Preliminary investigations of the local bats indicate that they also represent a potential reservoir of histoplasmosis. Soil samples have yielded two isolations of H. capsulatum.

ARBOR VIRUSES IN MIDDLE AMERICA

From the Pacora area in the Republic of Panama where cases of encephalitis in horses have been observed, sera from over 200 wild birds, about 100 wild bats, 55 human beings, and 65 horses have been collected. In addition a total of 2,500 human sera from the Republic of Panama have been collected for similar testing. Materials from most of the animals and birds were collected for the purpose of attempting viral isolation as well as more than 25,000 mosquitoes of at least 11 different genera. No isolation of EEE virus has been made from any source except the brains from the two encephalitic horses. Serological studies in progress so far disclosed antibodies against this virus in 44 of the 65 horses, possibly in three of the humans, and in none of the domestic fowl tested.

Studies of the sera from human beings in selected localities in Guatemala have indicated present or past activity of the viruses of St. Louis encephalitis, Ilheus, western equine encephalomyelitis, eastern equine encephalomyelitis and yellow fever. The populations of the lowland areas were especially involved whereas the residents of the mountainous areas were less affected. Studies of residents of the Republic of Panama yielded similar results. Tests on sera from Miami residents indicate the activity of St. Louis encephalitis, encephalomyocarditis, and possibly even Ilheus virus. Facilities for carrying out transmission studies on several clinically important tropical viruses are now in use.

At Bethesda the recently initiated studies in immunology and allergy have followed productive courses.

MECHANISM OF ALLERGIC REACTIONS

In man anaphylactic or anaphylactoid reactions following the injection of allergenic material are not uncommon and may be fatal. These reactions are prolonged more frequently than they are acute, and have not been studied experimentally to great extent. In the past anaphylactic shock was studied experimentally mainly in the guinea pig and after intravenous injection of antigen resulting in death within a few minutes after injection. Scientists in the Laboratory of Immunology regularly induced protracted anaphylactic shock in the guinea pig. Using this model of anaphylactic shock they made contributions to understanding of the mechanism of anaphylactic shock as it occurs in man.

Experimental "allergic" encephalomyelitis can be induced in rats by an injection of brain or spinal cord emulsified in complete adjuvant. This experimental disease was for the first time passively transferred by injecting "immune" lymph cells. The recipient rats had been made tolerant to the lymph cells of the donors by previous, neonatal injection of spleen cells of the donor rats. This and supporting evidence indicates that this experimental disease has an immunological mechanism. Other inquiries show that rats can be made tolerant to paralytogenic spinal cord adjuvant emulsions by injection at one day of age with spinal cord tissue. Brain material from 1-9 day-old rats has no demonstrable paralytogenic activity.

Joint researches with Dr. A. A. Miles, the Lister Institute of Preventive Medicine, London, have clarified and defined the vascular permeability factor found in a constituent of the guinea pig coagulation gland. The active fraction contains a recognized esterase and is sixteen times more potent than histamine weight for weight. On a molar basis it is 9,000 times more active than histamine.

Other studies showed that components of serum from individual rabbits are antigenic in other rabbits. Isoprecipitins can be produced which react with serum antigens having electrophoretic mobilities corresponding to the different globulins. Individual rabbits have one or both of the gamma globulin specificities in their serum.

Fluorescent microscopic methods demonstrated the deposition of tetracyclines in proliferating bone formations of certain bone tumors.

HOMOGRAFTS IN INBRED GUINEA PIGS

The availability of two strains of inbred guinea pigs afforded a well controlled examination of the immune mechanism of homologous skin grafts. These guinea pig strains are genetically identical to the extent of allowing permanent survival of skin grafts exchanged between individual animals within each strain, but not between individuals of the two different strains. Individuals of the F_1 hybrid will accept grafts from both parent strains. The failure of passive transfer of homograft immunity with "immune" serum adds evidence to the concept of the "cell fixed" noncirculating nature of homograft immunity.

NIAID
Scientific Planning and
Development

Estimated Obligations for FY 1959

Total:	$145,700
Direct:	112,800
Reimbursement:	32,900

Annual Report of Program Activities

National Institute of Allergy and Infectious Diseases
January-December 1958

Extramural Programs

Although five of the ten leading causes of morbidity and mortality are attributed to microbiologic and parasitic infections, and although it is estimated that more than half our population suffer from varying degrees of hypersensitivity, problems in the control of the communicable and allergic diseases continue to be seriously underestimated. Cognizant of this, the Institute has continued its emphasis on basic research designed to illuminate pathogenesis and host-parasite interrelationships and to contribute to prophylaxis and specific therapy. Tables I and II are indicative of the distribution of research funds in support of these objectives.

The year has been notable for the initiative which the Council has assumed on a number of perplexing problems and for the accomplishments of the Extramural Programs as an outgrowth of this initiative. Thus, under an agreement stimulated by the Council, the Communicable Disease Center is now producing, standardizing, and distributing non-commercially available diagnostic typing sera to Public Health Service grantees and other scientists who can justify the need. The Council has also taken the first practical step in thirty years towards the standardization of allergens. A small task force, supported by a $2,000 grant, has been authorized to explore the possibility of establishing a valid method for standardizing extracts of ragweed pollen and to consider the advisability of the standardization of other allergens in a similar manner.

In addition, the Council has focused attention on the magnitude of penicillin sensitization; has been instrumental in the establishment of a number of training centers in allergy, immunology, rickettsiology, mycology, and parasitology; and is taking steps to establish additional tropical medicine centers in key port areas of the country. Concomitantly, the National Research Council has been awarded a three-year grant for a survey of the national and international research status and needs in tropical medicine. Moreover, the Council has urged that increased attention be given to the promotion of Polar zone studies.

Troubled by the lack of factual data on the epidemiology of cystic fibrosis, the Council endorsed an appropriate investigation to be financed by the Institute and conducted by the Children's Bureau of the Public Health Service. In the meantime, support has been given for a conference to be held in January, 1958, under the

sponsorship of the National Cystic Fibrosis Foundation, the Arthritis and Metabolic Diseases Institute, and our Institute. Also, eight research grants for approximately $180,000 have been recommended.

Long familiar with the unabated increase in the number and in the severity of staphylococcal infections, especially in hospitals, the Council, thanks to a Congressional appropriation of $1,000,000 for the study of the organism and its ecology, has already recommended 59 proposals for nearly $850,000 and is confidently expecting to finance a far greater effort in the immediate years ahead.

Despite a substantial increase in research grant funds for fiscal year '59, there are currently about $1,000,000 of projects recommended for approval which cannot be financed.

Because of the increase in the training grant appropriation, support is being provided for the first time in general microbiology. In addition, a significant number of programs initiated last fiscal year at a minimal level for lack of funds were restored to their full, planned operating capacity.

One of the leading projects undertaken by the Training Grant Committee, under Council sponsorship, was an investigation into the feasibility of establishing centers for tropical medicine in key areas of the country. After visits had been made to eight such areas, a preliminary report was presented and a subcommittee was established to prepare a final report to the Council early next year.

To provide scientific coverage previously lacking, the Training Grant Committee was enlarged towards the end of the current year; furthermore, in anticipation of the desirability of dividing the Committee into two panels at a later date, authorization was obtained from the Surgeon General to establish an Allergy and Immunology and Infectious Diseases panel.

For the first time since its existence, the Institute was allocated sufficient funds - $605,000 - on which to base a sound fellowship program. An auspicious advance has been made towards the objective of increasing the number of post-doctoral and special fellows in all areas of major Institute responsibility.

FISCAL YEARS

Area of Support	'56 Appropriation* $2,110,000		'57 Appropriation* $8,212,000		'58 Appropriation* $10,824,000		'59 Appropriation* $14,880,000	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount (Estimated)
Allergy	1	$ 4,293	71	$1,091,324	92	$1,339,584	130	$1,800,000
Immunology	8	73,965	57	669,522	65	776,143	90	1,000,000
Bacteriology	56	677,884	164	1,847,267	213	2,594,836	300	3,650,000
Virology	45	615,165	104	1,736,694	146	2,701,257	170	3,100,000
All Others (Antibiotics, Arthropods, Biochemistry, Epidemiology, Genetics, Mycology, Parasitology, etc.)	153	1,283,786	351	2,947,130	407	3,752,420	510	4,699,000
Grand Total	263	$2,655,093**	747	$8,291,937**	927	$11,164,240**	1200	$14,249,000

* The '56 and '57 appropriations included $147,000 for Gorgas Memorial Laboratory, and the '57 also $21,000 for Russian Translation Service; the '58 and '59 appropriations include $150,000 for Gorgas and $18,000 and $39,000 respectively for Russian Translation Service. In addition, $442,000 was deducted from the '59 appropriation to cover NIAID salary increases.

** Excess listed over appropriation represents extensions without additional funds and grants, paid from previous year fiscal funds, which will terminate during current fiscal year.

SPECIAL AREAS*

Area of Support	'56	Appropriation	'57	Appropriation	'58	Appropriation	'59 (Estimated)	Appropriation
1. Acute Respiratory Disease (Airborne Allergy, Common Cold, Influenza, Ornithosis, Pneumococci, Q Fever, Viral pneumonia)	18	$ 259,933	42	$ 739,844	58	$1,153,109	96	$2,000,000
2. Cystic Fibrosis	-	- -	4	35,700	4	36,511	11	160,000
3. Parasitology and Tropical Medicine:								
Sub-total	122	1,073,901	263	2,240,036	284	2,579,744	327	3,000,000
Parasitology:								
Helminths	36	333,723	59	459,136	67	592,196	73	650,000
Protozoa	33	251,119	67	565,640	64	566,072	73	650,000
Entomology (Arthropods):	24	165,841	57	419,006	60	496,189	66	560,000
Arthropod-borne Viral Diseases:								
Dengue	1	13,133	1	14,641	2	37,732	2	28,000
Encephalitis	2	20,137	3	68,392	5	99,955	9	140,000
Yellow Fever	-	- -	-	- -	1	32,545	1	28,000
Non-Parasitic Tropical Diseases of Importance:								
Cholera	-	- -	1	10,350	1	7,360	2	20,000
Leprosy	3	40,950	4	72,824	7	78,709	9	104,000
Salmonellosis	-	- -	4	33,235	6	65,225	10	100,000
Shigellosis	2	48,682	5	51,416	2	20,126	6	60,000
Viral Dysentery	3	49,846	5	93,802	3	39,828	5	60,000
Mycology	18	150,470	57	451,594	66	543,807	71	600,000
4. Staphylococci:	2	19,440	10	102,699	19	189,573	60	1,050,000
5. Tuberculosis:	13	210,390	34	524,744	44	694,979	64	958,791

*(Included in Disciplines on first page.)

I. RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1958

	Received		Approved	
March	No.	Amount	No.	Amount
New	139	$1,538,630	84	$ 845,936
Continuations	79	1,053,271	58	688,595
	218	2,591,901	142	1,534,531
June				
New	187	2,388,883	118	1,483,544
Continuations	694*	9,538,998	677*	9,191,147
	881	11,927,881	795	10,674,691
November				
New	181	2,969,031	107	1,555,027
Continuations	79	1,236,411	66	973,507
	260	4,205,442	173	2,528,534

(*Includes all Continuations with commitments, considered at the June meeting (597 for $8,154,557)

II. TRAINING GRANTS

	Received		Approved	
	No.	Amount	No.	Amount
March				
New	10	$225,477	5*	$ 72,228
June				
New	21	$787,125	15	$467,464
Continuations	26	475,506	26	475,506
November				
New	10	$322,372	8	$211,068

Distribution of Support

Area	No. of Grants
Allergy and Immunology	8
Parasitology and Tropical Medicine	8
Mycology	1
Rickettsiology	0
Microbiology	10
	27

* 1 in the amount of $17,349 was disapproved at June 1958 Council due to change in Program Director.

III. FELLOWSHIPS (Fiscal Year 1959 appropriation $605,000)

Fellowship applications considered by the NIAID Board	176
Fellowship applications approved by the NIAID Board	164
Awarded by other Institutes	1
Awarded by NIAID	75

By Types

pre-doctoral	53
post-doctoral	21
special	1
	75

By Categories

Allergy	4
Amebiasis	1
Antibiotics	5
Arthropods	3
Bacteriology	5
Biochemistry	17
Brucellosis	2
Coccidiosis	1
Diphtheria	1
Epidemiology	1
Fungi	3
Genetics	1
Helminths	1
Herpes Simplex	2
Histamine	2
Immunology	3
Influenza	1
Moniliasis	1
Physiology	2
Plague	1
Public Health and Sanitation	1
Tapeworm	1
Trematodes	2
Tularemia	1
Viruses	13
	75

Accomplishment Highlights

The growth of the extramural programs is represented by the pyramiding of research accomplishments reported by grantees of this Institute. Some of the most important discoveries are related to basic processes rather than specific diseases. These are the foundation stones upon which future investigations, unlimited in variety and scope, will be built. The accomplishments which follow have been selected to give some indication as to the wide range of interests and responsibilities within the purview of this Institute.

Investigators at the California Institute of Technology have discovered that certain antigens injected into rabbits will persist in the liver for months and even years. Since antigens are a source of antibody formations in the body, these may give an extended immunity or may cause a prolonged allergic reaction. In the latter case a means can be found to flush out the antigen and eliminate the sensitivity. It is believed that persistent antigens may also be involved in immune tolerance, anamnestic or secondary responses and perhaps the stabilization of small amounts of antibody tissue spaces.

A grantee at the Alabama Polytechnic Institute has discovered what is the first systemic insecticide capable of eliminating some disease-carrying parasites. A compound, Dow-ET-57, when administered in the food of cattle will circulate among body fluids and destroy the common cattle grubs which have burrowed into the flesh. This insecticide is being marketed in certain areas of Iowa, Nebraska, South Dakota, and Wyoming. Other compounds are being tested as systemics against disease-spreading, blood-sucking ectoparasites such as the stable fly, the Gulf-Coast tick, the bed bug, the Lone Star tick, and the yellow fever mosquito.

Plasmapheresis is a method through which, according to investigators at the Children's Hospital, Philadelphia, a person may safely donate blood plasma up to 50 times a year. Results of employing plasmapheresis on volunteer donors have now been observed for more than two years by these grantees. Two donors gave weekly and 23 gave blood plasma bi-weekly for 40 weeks. The fractionator employs a closed system centrifugation principle. The lighter plasma component of blood is retained and sealed in plastic bags; the heavier part, including the red and white cells, is put back into the donor almost immediately. Plasma proteins are regenerated in the donor within 16 to 18 hours to replace those withdrawn.

Two investigators at the Children's Medical Center in Boston have conducted long term studies supported by the National Institute of Allergy and Infectious Diseases and the National Institute of Arthritis and Metabolic Diseases, which include diagnostic methods, therapeutic measures and clinical evaluations of cystic fibrosis of

the pancreas. 105 patients were followed for 5 to 14 years; of this number 95 are still living. This does not substantiate the widely held belief that cystic fibrosis is inevitably a fatal disease in infancy or early childhood. The effect of pulmonary involvement reflects the severity of cystic fibrosis, as the cause of death is eventually pulmonary insufficiency from a combination of diffuse obstructive phenomena and infection. The investigators stress the effectiveness of the medical regimen employed and emphasize the value of pursuing a vigorous therapeutic program in a disease which is still so little understood.

Three investigators at Northwestern University have used a new steroid hormone having twice the potency of prednisone for the treatment of allergic diseases, such as asthma, hay fever, urticaria, and a variety of dermatoses. The investigators report that the new drug - triamcinolone - behaves like other anti-inflammatory drugs with respect to side effects except that there is a marked diuresis and sodium removal. The investigators consider the sodium removal a most advantageous feature in treating patients with cardiac disease or hypertension.

Our knowledge of how an antibiotic inhibits and how an organism overcomes this inhibition lags far behind the discovery and use of antibiotics. Staphylococcal resistance is today one of our gravest medical problems and, since investigators can easily produce, in vitro, strains of many bacteria resistant to all antibiotics, other forms of resistance will probably emerge in the future. This Institute is contributing to the support of a project at the University of Oklahoma which seeks to discover how resistance arises and how it can be prevented. Resistance to antibiotics is usually attributed to spontaneous mutation or adaptation, but this study shows that Staphylococcus aureus builds a resistance to low concentrations of chloramphenicol through adaptation and responds to high concentrations with mutation. The studies suggest further that adaptive resistance occurs because of the presence of the antibiotic and is more permanent than the non-specific resistance resulting from cellular metabolism and growth.

At Louisiana State University a grantee has demonstrated a highly effective treatment for round worm infections. He reports that dithiazanine eliminated round worm infections in 89% of the patients treated. In an earlier study supported by this Institute, the investigator found dithiazanine effective in the treatment of patients with either single or multiple intestinal helminth infections such as whipworm, threadworm, roundworm, and pinworm.

Seeking a key to the process undergone by the tubercle bacillus an investigator at the University of Pennsylvania made a study of the

interplay between antibodies and antigens in the immune mechanism. The report records tests of different serums against BCG tubercle bacillus which show that antibodies have little direct inhibitory effect against this organism. A substance, lysozyme has been found to have an inhibitory effect on the growth of the tubercle bacilli by attacking one of its components. Experiments are under way now to induce the production of antibodies by vaccinating animals natively resistant to tuberculosis.

Although treatment of allergy with a tranquilizer is generally unsatisfactory some investigators at the Northwestern University report singular success in the relief of chronic urticaria (hives) and dermographia (a condition in which the skin is peculiarly susceptible to irritation) by the use of hydrozyzine (Atarax). Most striking results were observed in patients with chronic urticaria of undetermined cause. 15 of 17 patients experienced complete relief and the remaining 2 showed continuing improvement.

NIAID
Review and Approval

Estimated Obligations for FY 1959

Total:	$304,000
Direct:	173,000
Reimbursement:	131,000

1958 ANNUAL REPORT
LABORATORY OF CLINICAL INVESTIGATION
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Research in clinical problems during calendar year 1958 continued previous programs in respiratory viral diseases, mycotic infections, and parasitic diseases. Principally, these investigations resulted in new knowledge and laid the basis for future clinical research of a more intensive nature.

During the year the number of patients studied increased and the number of patient days also increased nearly fifty percent over 1957. There was less emphasis than in former years on bacterial diseases and more effort in problems related to viral infections. These included investigations of aseptic meningitis due particularly to mumps and ECHO 9 viruses. Parasitic infections were similarly emphasized, both from a standpoint of patient care and supportive laboratory procedures. Cooperation with the scientists of the Laboratory of Tropical Diseases permitted more comprehensive and detailed clinical investigation.

Problems of staphylococcal infections, especially those due to antibiotic-resistant strains predominated among bacterial diseases investigated both at the bedside and in the laboratory. Studies involved therapeutic investigations of various drugs, antibiotics, and the management of the patients' illness. We began, in addition, to explore the mechanisms by which staphylococci develop pathogenicity for animals and resistance to antibiotics. Cystic fibrosis also received particular attention, especially in the management of complications caused by staphylococci or gram-negative bacteria. Studies on the streptococcal infections and brucellosis received diminished emphasis as did other bacterial infections for which there has been little opportunity for investigation.

The increasing necessity for incorporating into clinical investigation the new knowledge and techniques emerging from basic research laboratories becomes more evident each day. Means of making these skills available to clinical investigation have been explored. Plans have been laid for further development of such cooperative studies as vaccine trials new drug regimens and more elaborate observations on the effect of new viruses in man. This increases the responsibilities of the clinical investigator in the care and management of such patients but also increases the opportunities for clinical studies opened up by nonclinical research on agents of disease.

On July 1, 1958, Dr. John P. Utz assumed duty as Acting Chief with the reassignment of Dr. Norman B. McCullough as Chief, Laboratory of Bacterial Diseases. Dr. Henry K. Beye resumed duties as Chief Parasitic Diseases Service, after a year of clinical experience at a teaching institution.

Major Research Accomplishments

Mumps continued to be a major subject of investigation. It offers excellent opportunities for application of recent viral techniques to a well defined disease. One aspect of interest has been the finding of virus in urine of patients with mumps. The excretion of virus in urine occurred as early as the first day and as late as the fourteenth day of illness. Improved techniques developed for the detection of virus revealed for the first time in human urine five other viruses: ECHO 9, Coxsackie B2, B3, B4, and a new Enterovirus. This new approach may open up avenues for investigation of nephritis, pyelonephritis and other diseases of the kidney. It may also provide ways of diagnosing viral infections.

During 1958 the Washington area experienced an outbreak of aseptic meningitis and 19 patients came to the Clinical Center for intensive study. Of these patients 12 suffered from an infection with ECHO 9 virus. This virus occurred in the blood and urine of a number of these patients. Studies on three other patients yielded other viruses. These investigations afforded the opportunity for careful correlation of clinical findings with the virologic and serologic observations. As an outgrowth of the clinical observation of viruria, investigators initiated studies in monkeys and other animals on experimental viruria.

The outbreak of aseptic meningitis, associated principally with the ECHO 9 viruses, recalls the experience in 1957 in this area. In that year aseptic meningitis was due principally to poliovirus III. This study is of interest, in that it showed the highest attack rate in the United States in 1957 and a marked selectivity of paralysis for preschool age children within the lower socio-economic category. This is of special importance in pointing out to health officials that part of the population both most susceptible to infection and least likely to have been vaccinated in present day immunization programs.

As more and more antibiotic drugs become available for bacterial infections, increasing attention is being paid to the large group of fungal infections still, for the greatest part, incurable. Hence, the evaluation of Amphotericin B in this laboratory is an important research project. Studies on 25 patients with culturally proved and commonly fatal systemic fungal infections have shown that Amphotericin B is the only effective antibiotic thus far for histoplasmosis. In addition, Amphotericin B has been demonstrated to be superior to any other drug available for treatment of blastomycosis. The role it will play in cryptococcal infections is in doubt at present. Coccidioidomycosis remains as the major fungal disease unaffected thus far by known antibiotics and should receive increased study.

Studies of fungal infections have led us further into the broad category of granulomatous diseases in general. In one of these, sarcoidosis, the myriad of clinical aspects, has prompted the hope that some key may be found in one of them that will explain the basic pathology of this group of diseases. Studies on one such aspect, calcium metabolism, have continued and have shown multiple abnormalities strikingly akin to Vitamin D intoxication. In another aspect, hypoferremia and increased iron turnover rate have been demonstrated for the first time in over 10 patients.

Work on the Parasitic Disease Service has led to the development of a new method of treating Taenia saginata and Taenia solium infestations. Atabrine administered through a duodenal tube has been successful in eradicating tapeworm in 14 consecutive patients, and this group is now large enough to serve as a control for other drugs that can be administered without a tube.

Basic work continued in applied immunology, where it has been possible to produce in the kidneys of guinea pigs and rabbits hematoxyphil lesions that are identical in every respect to those found in the kidneys of patients with lupus erythematosus. This was accomplished by the development of a new technique of repeated intra-aortic injection by percutaneous catheter of suspended L. E. cells and L. E. bodies obtained from patients with this disease. Studies continued, in addition, on the effect of steroids on antibody response, tolerance to glucose, changes in respiratory function, effect on dissemination of fungi, and changes in amount of L. E. material.

Finally, this year saw the initiation of two new investigative approaches to cystic fibrosis, designed to determine whether protein may be lost in the intestinal tract, and to define further by chemical and antigenic means the abnormal factor present in the mucus of these patients.

NIAID
Laboratory of
Clinical Investigations

Estimated Obligations for FY 1959

Total:	$1,509,500
Direct:	466,500
Reimbursement:	1,043,000

Covering Project Reports
NIAID....10(c) thru 25(e)

Form No. ORP-2
November 1958

Serial No. NIAID - 10 (c)
1. LCI
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Office of the Chief

Principal Investigator: Dr. Norman B. McCullough (To July 1, 1958)
Dr. John P. Utz (July 1 - Present)

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):		Patient Days (calendar year 1958):
Total:	4.2	None
Professional:	.2	
Other:	4.0	

Project Description:

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to re-direct individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

Part B included Yes ☐ No ☒

Form No. CRP-2
November 1958

Serial No. NIAID - 11 (c)
1. LCI
2. Infectious Disease & Pediatric Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Rheumatic Fever and Streptococcal Infection

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Norman B. McCullough (to July 1, 1958) and Dr. Charles B. Neal

Cooperating Units: Laboratory of Infectious Diseases, NIAID

Man Years (calendar year 1958): Patient Days (calendar year 1958):

Total:	0.4
Professional:	0.4
Other:	0.0

377

Project Description:

A. Rheumatic Fever

Objectives:

To evaluate and establish clinical and laboratory criteria for diagnosis. To study pathogenesis of the disease, particularly the factors that lead to cardiac involvement, and experimental therapy.

Methods Employed:

Clinical studies only. Correlation of various biochemical tests with clinical observations. Cooperation with other units of NIAID.

Patient Material & Major Findings:

None - due to lack of patient material.

Significance to Microbiological Research:

Rheumatic fever is a major unsolved public health problem. Aids to definitive diagnosis are badly needed.

Proposed Course of Project:

This project will be pursued if and when patient material becomes available.

B. Streptococcal Infection

Objectives:

To determine the course and sequellae of this disease in children. To establish guidelines for therapy. To establish guidelines for the antibody therapy of subacute bacterial endocarditis, particularly that due to Group D streptococci.

Methods Employed:

Glomerulonephritis: Clinical studies only.

Subacute Bacterial Endocarditis: Standard laboratory tests for determining antibiotic sensitivities of causative organisms; determination of serum antibiotic levels; use of various antibiotic regimens.

Patient Material & Major Findings:

Series is too small for definite conclusions.

Significance to Microbiological Research:

The pathogenesis of glomerulonephritis is unknown. The disease is common and sequellae are serious. The disease lends itself to the study of basic immunologic phenomena. The therapy of subacute bacterial endocarditis due to Group D streptococci is often unsuccessful and effective therapy is needed.

Proposed Course of Project:

Studies to be pursued when clinical material is available.

Part B included Yes[ ] No[X]

Form No. OPR-2
November 1958

Serial No. NIAID-12(c)
1. LCI
2. Infectious Disease & Pediatric Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: **Respiratory Viral Diseases**

Principal Investigator: Dr. John F. Utz

Other Investigators: Dr. Leon G. Smith, Dr. Charles B. Neal, Julius A. Kasel, and Clarence F. Sawed

Cooperating Units: Laboratory of Infectious Diseases, NIAID
U.S. Naval Medical Center, Bethesda, Md.
Mt. Alto Hospital, Washington, D. C.

Man Years (calendar year 1958):

Total:	3.7
Professional:	1.7
Other:	2.0

Patient Days (calendar year 19

1,477

Project Description:

Objectives:

1. To define clinical entities in relation to newly isolated respiratory viruses.

2. Further to define diagnostic criteria, pathogenesis immune response, persistence and sites of virus and effects on tissues of virus in certain infections of respiratory passages and mouth.

3. To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory diseases.

4. To improve clinical laboratory techniques in the laboratory confirmation of respiratory viral disease diagnosis

Methods Employed:

Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolation employing both animals and tissue culture techniques.

Patient Material and Major Findings:

(1) The Diagnostic Virology Unit examined a total of 1781 specimens from Clinical Center in-and-outpatients. Virus was isolated in 258 of these specimens achieving etiologic confirmation of the diagnosis in a total of 127 patients.

(2) Clinical and laboratory studies were performed on a relatively small number of patients ill with Asian influenza.

(3) Excretion of virus in urine of patients with mumps has been further delineated. Virus is present in urine as early as the first day of clinical illness and as late as the 14th day in some patients. Before the end of the last mumps epidemic, the renal function in a few patients was studied. These studies will be further pursued with the next outbreak.

(4) Viruses isolated recently by new tissue culture techniques from patients have been authenticated as mumps by a number of studies including the ultimate feasible test of the production of mumps infection in two monkeys.

(5) A new viral agent has been found in stools of patients with an otherwise undifferentiated upper respiratory illness. This virus has the characteristics of the Enterovirus Group with the heretofore unique property of not propagating in monkey kidney cell tissue culture or in suckling mice.

Significance to Microbiological Research:

Illustrates progress in study of acute and chronic respiratory diseases, which are prime unsolved problems in the infectious disease field. The major advances in this field are being made in settings such as this where the efforts of research laboratories in bacteriology, mycology and virology can be correlated with clinical material under ideal study conditions.

A major effort is exerted toward clinical studies in relation to newer viruses uncovered by tissue culture techniques.

Proposed Course of Projects:

Studies will continue as outlined under "Objectives" in patients referred to the Clinical Center and in other hospital or community surveys when indicated. This project should be expanded because of the great importance of careful clinical studies on patients with respiratory illnesses in order to link these illnesses with viral agents that are now being isolated in a number of laboratories.

Part B included Yes [x] No [ ]

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1.* Rowe, W. P., Hartley, J. W., Cramblett, H. G., and Mastrota, F.M.: Detection of human gland virus in the mouth and urine of children. Am. J. Hyg. 67: 57-65, 1958.

2.* Cramblett, H. G., Rosen, L., Parrott, R. H., Bell, J. A., Huebner, R. J., and McCullough, N. B.: Respiratory illness in six infants infected with a newly recognized ECHO virus. Pediatrics. 21: 168-177, 1958.

3.* Merchant, R. K., Rowe, W. P., Kasel, J. A., and Utz, J. P.: Pharyngoconjunctival fever due to type 1 adenovirus. New Engl. Jour. Med. 258: 131-133, 1958.

4. Utz, J. P., Szwed, C. F., and Kasel, J. A.: Clinical and laboratory studies of mumps. II. Detection and duration of excretion of virus in urine. Proc. Soc. Exper. Biol. & Med. 99: 259-261, 1958.

5. Utz, J. P.: The laboratory diagnosis of respiratory viral disease. GP. XVIII: 90-92, 1958.

6. McCullough, N. B., and Cramblett, H. G.: Respiratory viral diseases. GP. XVIII: 93-97, 1958.

7. Kasel, J. A., Cramblett, H. G., and Utz, J. P.: Agents resembling the enteroviruses isolated in HeLa cell cultures from fecal specimens. Proc. Exper. Biol. & Med. (in press)

* Listed in last year's report as "in press".

Form No. ORP-2
November 1958

Serial No. NIAID - 13 c:
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS, NIAID
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Brucellosis

Principal Investigator: Dr. Norman B. McCullough

This project was transferred to the Laboratory of Bacterial Diseases on July 1, 1958, and will be submitted by that Laboratory.

Form No. OPR - 2
November 1958

Serial No. NIAID - 14(c)
1. LCI
2. Bacteriology Section
3. Bethesda Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Staphylococcal Diseases

Principal Investigator: Miss Rose Lieberman

Other Investigators: Dr. Bayard S. Tynes

Cooperating Units: None

Man Years (calendar year 1958):		Patient Days (calendar year 1958):
Total:	1.8	
Professional:	0.8	1,093
Other:	1.0	

Project Description:

Objectives:

Basic studies of the etiology, pathogenesis, and immunology of Staphylococcal diseases which include:

1. Studies of the relations that exist between the cultural characteristics and the pathogenic properties of Staphylococcus.

   a) Correlation of *in vitro* and *in vivo* studies of components and products of Staphylococcus that are responsible for their ability to establish infection.

   b) Epidemiological study of Staphylococci isolated from clinical cases including serological and bacteriophage classifications and measurements of drug resistance.

   c) The quantitative estimations of components and products of Staphylococcus contributing to its pathogenesis.

   d) Developments of new techniques that would provide information concerning the mechanisms involved in the establishment of Staphylococcal infections.

2. Studies of acquired immunity to Staphylococcal infections.

   a) Studies of environmental factors of the host which permit the Staphylococcus to develop intra- and extracellularly in the presence of antimicrobial and other defense mechanisms.

   b) Studies of the changes in the immune state of the host or physiological factors that may be responsible for the initiation of the infective process.

   c) Studies of non-specific factors that affect susceptibility to Staphylococcus infections including nutrition, metabolic disturbances, toxemias and allergic states.

   d) Studies of tissue environment that may determine the outcome of the infection as vascularity, inflammation and biochemical reactions at site of the infection.

3. Clinical study of Staphylococcal infected patients and evaluation of new antimicrobic drugs.

Methods Employed:

1. Study of specific bacteriophage strains exhibiting antimicrobial resistance that have been isolated from patients with Staphylococcal infections including:

   a) Determination of serological components of specific bacteriophage strains.

   b) The growth of specific strains under different environmental conditions to determine what serological components are produced.

   c) Immunization of animals with different serological components of antigens to determine the protective effect of each component.

   d) The study of the effects on protection of the fractionation, separation and combination of specific Staphylococcal antibodies.

2. Study of the effect of pure coagulase on the phagocytosis of Staphylococcal using in vivo and in vitro techniques.

   a) Differences in the phagocytosis of specific Staphylococci by "immune" and normal WBC.

   b) Differences in the infectivity of intra-and extracellular Staphylococci in the presence and absence of coagulase.

3. Production of ascites in mice by Staphylococcus to provide an excellent source of supply for large amounts of potent antibody to be used for study.

   a) Ascitic fluid containing Staphylococcal antibodies is used to measure the protective effects of various antibody components in passive immunization of mice.

   b) The protection endowed by ascitic fluid containing antibodies in the presence of properdin, complement, or normal serum is estimated in passive immunization tests in mice.

   c) The differences between agglutinins and protective antibodies in serum are compared with those found in the ascitic fluid.

Major Findings and Patient Material:

Under a definite immunization regimen, Staphylococcus has induced the formation of ascitic fluid containing higher titers of antibodies in 100% of the mice treated. A manuscript presenting the above data is now in press.

The Staphylococcal antibodies thus produced are being fractionated and studied alone, in combination with each other, and in combination with normal and immune serum.

The growth of Staphylococcus under different environmental conditions produce antigens which appear to block the antigens that appeared in the earlier stage of growth. The immunological significance of these antigens are being studied by absorbing Staphylococcal immune serum with these antigens and then studying the effects on protection, agglutination, infectivity, etc.

Significance to Microbiological Research:

The increase in number of primary Staphylococcal infections, as well as the increased number of "Hospital" infections with specific bacteriophage antibiotic resistant strains has become an important public health problem. There is no successful therapy for the treatment of these diseases although isolated cases respond to different antimicrobial and surgical therapy.

The basic antimicrobial and epidemiological problems studied under this project, may contribute much to the understanding of the etiology of Staphylococcal diseases.

Proposed Course of Project:

To continue as outlined under objectives.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Lieberman, R., Douglas, J. O. A., and Humphrey, W., Jr.: Ascites induced in mice by staphylococcus. Science. (in press)

Form No. CRr-2
November 1958

Serial No. NIAID-15(c)
1. LCI
2. Infectious Disease & Pediatric Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hepatitis and Mononucleosis

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. James Kirby

Cooperating Units: None

Man Years (calendar year 1958):		Patient Days (calendar Year 1958):
Total:	0.4	
Professional:	0.4	619
Other:	None	

Project Description:

A. Infectious Hepatitis

Objectives:

1. Better to define the diagnosis, differential diagnosis and natural course of infectious hepatitis and homologous serum hepatitis.

2. To investigate factors which predispose to chronic hepatitis or post hepatic cirrhosis.

Methods Employed:

Clinical observation and clinical laboratory data are compiled on Clinical Center patients. Family contacts are examined and specimens obtained for clinical laboratory procedures in an attempt to define subicteric infection. Similar liver function tests are obtained on most of the febrile patients admitted to the Service for control and for evaluation of hepatitis in other entities. Appropriate procedures are employed to assess the role of other organisms such as leptospira and bacteria in hepatitis.

Patient Material and Major Findings:

A few patients have been hospitalized and are being followed in long-term studies to determine the value of such treatment measures such as bed rest. Newer diagnostic tests such as isocitricdehydrogenase determination are similarily being evaluated.

Significance to Microbiological Research:

The clinical spectrum of infectious hepatitis is not well defined by objective methods, but apparent infection is responsible for serious illness and death in all age groups. The frequency and clinical nature of subicteric infection is not known, but on the basis of high gamma globulin protective ability, a majority of adults have been exposed to the causative agent. This disease constitutes a major public health problem.

Proposed Course of Project:

To continue as outlined under "Objectives" and "Methods" on larger numbers of patients.

B. Infectious Mononucleosis

Objectives:

To assess the degree of involvement especially that in the liver of the human patient with infectious mononucleosis; to attempt virus isolation from patients during various periods of their illnesses using fresh human tissue culture techniques; to determine metabolic and immunologic changes in patients with severe generalized viral disease; and to investigate for other than viral causes of this syndrome.

Methods Employed:

Hospitalization of patients for study by clinical observation and laboratory methods. Laboratory methods involve primarily the newer tissue culture techniques, such as attempts to unmask viruses by the Huebner-Rowe technique.

Patient Material and Major Findings:

None. Too few patients are being seen to accumulate data.

Significance to Microbiological Research:

Infectious mononucleosis is a common and perhaps even universal infection of man. Certain characteristics of the disease suggest that it may be an intermediate link between infections and neoplastic blood dyscrasias. Since heterophile titers develop, the disease lends itself to basic studies in immunology.

Proposed Course of Project:

This study is temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Part B included Yes ☐ No ☒

Form No. ORP-2
November 1958

Serial No. NIAID - 16(c)
1. LCI
2. Infectious Disease & Pediatric Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Aseptic Meningitis

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Bayard S. Tynes, Dr. George M. Owen, Julius A. Kasel, and Clarence F. Szwed

Cooperating Units: Laboratory of Infectious Diseases, NIAID, and Children's Hospital, Washington, D. C.

Man Years (calendar year 1958):		Patient Days (calendar year 1958)
Total:	3.8	
Professional:	1.8	590
Other:	2.0	

Project Description:

Objectives:

a) To develop methods and use newly available methods to diagnose the large group of non-bacterial meningo-encephalitides.

b) To correlate etiological findings with detailed historical, physical and clinical laboratory observations.

c) To further define existing etiological entities and to discover new etiological entities in this group of diseases.

Methods Employed:

Hospitalization of patients for clinical observation and laboratory studies. Laboratory procedures include: a) tissue culture and suckling mouse inoculation of material for virus isolation; b) appropriate serological procedures with acute and convalescent serum for the known viral meningo-encephalitides, Leptospira, Toxoplasma and infectious mononucleosis. A number of patients selected from other hospitals or surveyed groups will be studied similarily.

Patient Material and Major Findings:

1. A broad multifaceted study was made of the 1957 outbreak of aseptic meningitis in the Washington area. Thru studies at the Diagnostic Virology Unit, the predominant etiology of this outbreak was proved to be poliovirus III. This outbreak was unique in having the largest metropolitan attack rate observed in USA in 1957 and in showing a marked selectively of paralysis for preschool children within the low social-economic category. This study is to be published shortly.

2. In 1958, a cooperative study at Children's Hospital was undertaken again to study clinically and virologically the aseptic meningitis cases. Nineteen patients were admitted to the Clinical Center, fifteen had virus isolated, and twelve patients had ECHO 9 infection. A total of over seventy patients infected with this virus were studied along with other less numerous infections. ECHO 9 was isolated from the blood and urine of some of these patients.

3. The studies on a patient with extensive infection with Coxsackie B-3 were published.

Significance to Microbiological Research:

Non-bacterial meningitis and meningo-encephalitis is a common and often serious disease of whose etiology and pathogenesis there is insufficient knowledge. It often results in brain damage and behavior disturbances. Increased knowledge may also clarify the pathogenesis of the encephalitic complications of the otherwise minor diseases of children.

Proposed Course of Project:

Continue above studies.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Utz, J. P.: Coxsackie B virus infection - Presence of virus in blood, urine, and cerebrospinal fluid. J.A.M.A. 168: 264-267, 1958.

Form No. ORP-2
November 1958

Serial No. NIAID - 17(c)
1. LCI
2. Biochemistry & Immunochemistry Sect.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Allergy-Immunology

Principal Investigator: Dr. Norman B. McCullough (To July 1, 1958)
Dr. John P. Utz (From July 1, 1958)

Other Investigators: Dr. James L. German (To July 1, 1958)
Dr. John P. Masou (From July 1, 1958)

Cooperating Units: Laboratory of Infectious Diseases, NIAID;
The Laboratory of Immunology, NIAID; and
The Laboratory of Biochemistry, NCI.

Man Years (calendar year 1958):		Patient Days (calendar year 1958):
Total:	2.1	
Professional:	1.5	
Other:	0.6	1,866

Project Description:

Objectives:

1. To evaluate the role of immune serum globulins in host response to infectious diseases.

2. To evaluate the role of bacterial, fungal, or viral allergies in recurrent or chronic respiratory disease.

3. To determine the rate of metabolism of individual antibodies compared to that of gamma globulin, and to investigate the mechanism whereby the reticulo-endothelial system differentiates between otherwise chemically homogenous specific antibodies.

4. To measure, quantitatively, the rate of synthesis of specific antibody in the human during the primary and secondary immune responses.

5. To measure quantitative differences in the specific antibody response of acute leukemics, normal human volunteers, hypo-and hypergammaglobulinemias.

6. To determine whether, in the presence of a specific antigenic challenge, the specific antibodies are selectively removed from the total exogenous gamma globulin injected into agammaglobulinemia patients.

7. By accurately studying the true decline rate of injected gamma globulin in a large series of agammaglobulinemic patients, to arrive at an average figure to be used in the routine clinical management of such patients.

8. To determine the relationship of hormones to the gammaglobulinemic state.

9. To determine the pathogenic significance of the abnormal gamma globulin of patients with systemic lupus erythematosus.

10. To reproduce lupus lesions in experimental animals.

Methods Employed:

Clinical studies supplemented with:

1. Plasma protein analysis by (a) electrophoresis both by paper chromatography and moving boundary technique, (b) chemical determinations, and (c) immunologic assays.

2. Immunization and testing of response by serologic techniques or by challenge.

3. Antibody and total gamma globulin alteration measurement in agammaglobulinemic patients following hormone administration.

4. Pathologic study of animals following injection into the circulating blood of suspensions of L. E. cells and L. E. bodies.

5. Phase contrast microscopic study of the L. E. cell phenomenon.

Patient Material and Major Findings:

1. Clinical studies of the patients with lupus erythematosus and with agammaglobulinemia have continued.

2. A previously undescribed clinical syndrome characterized by recurrent febrile disease, chiefly lower respiratory; hepatosplenomegaly; anemia; leukocytosis; abnormal but not markedly depressed gamma globulin levels, has been observed and is being studied further.

3. Studies are continuing of the effect of steroids on a variety of patient responses. These include antibody rises, tolerance to glucose, changes in respiratory function, effect on dissemination of fungi, changes in amount of L. E. material, etc.

4. Hematoxylin bodies resembling in all ways those found in kidneys of lupus patients have been produced in glomeruli of rabbits and guinea pigs by intraaortic injection of suspensions of L. E. cells and L. E. bodies. By virtue of the size of these structures (observed through the phase-contrast scope to be larger than any normally circulating formed elements) they were apparently segregated in the glomerular vessels.

Significance to Microbiological Research:

This project exemplifies the use of clinical material in basic immunologic research. Knowledge resulting from such studies may be expected to increase our understanding of the infections and allergenic diseases and ultimately lead to improved clinical management of patients with such diseases.

Proposed Course of Project:

Study of the optimum methods of clinical management of agammaglobulinemic patients, especially as to the required frequency and dosage of gamma globulin injections and the antibiotic management, will be continued.

The immune mechanism of agammaglobulinemic patients will be further studied, especially as to their response to hormone administration.

Further expansion and integration with clinical and laboratory studies of Laboratory of Immunology.

Part B Included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

*1. German, J. L., and Huber, M.: Frozen cells as nuclear source in the L. E. cell phenomenon. Proc. Soc. Exper. Biol. & Med. 97: 221-223, 1958.

2. German, J. L.: Studies in the pathogenesis of lupus erythematosus; experimental production of hematoxyphil bodies in the kidney. J. Ex. Med. 108: 179-188, 1958.

3. German, J. L.: The glucose tolerance test after cortisone administration in obese and nonobese men. Diabetes. 7: 261-266, 1958.

*4. Utz, J. P., Frei, E., III, and McCullough, N. B.: Fever, infection and host resistance in acute leukemia. Am. J. Med. XXIV: 25-39, 1958.

* Listed in last year's report as "in press".

Form No. OFR-2
November 1958

Serial No. NIAID - 18(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Enteric Diseases

Principal Investigator: Dr. Norman B. McCullough (To June 30, 1958)
Dr. John P. Utz (From July 1, 1958 to present)

Other Investigators: Miss Rose Lieberman and Dr. Bayard S. Tynes

Cooperating Units: None

Man Years (calendar Year 1958):
Total: 2.0
Professional: 1.0
Other: 1.0

Patient Days (calendar Year 1958): 949

Project Description:

Objectives:

Basic studies of the etiology, pathogenesis, and immunology of enteric diseases which include:

1. The evaluation of antibiotic, immunologic and surgical therapy on the "carrier state" of Salmonellosis.

2. Study of the effects on protection of the fractionation, separation and combination of specific Salmonella antibodies.

3. Study of the structure of specific Salmonella antibodies in "resting" immune animals challenged with heterologous organisms.

4. The determinations of the effect on protection of combining specific antibodies of B. proteus.

5. The comparison of the effect of booster challenges of homologous versus heterologous antigens on the rise of titer of specific antibodies and on the duration of specific immunity.

6. Studies of cellular and humoral mechanisms in active Salmonella infections as compared to those present in the "carrier state."

Clinical studies: To investigate the factors that produce persistence of bacteria in some patients to test new antibiotics in such infections.

Methods Employed:

1. Complete bacteriologic and immunologic survey of enteric disease study patients.

   a) In vitro determinations of synergistic, or additive effects of combinations of antibiotics on organisms isolated from "carriers."

   b) Determination of gamma globulin, electrophoratic pattern, and agglutination in acute and chronic enteric disease states.

   c) Cholecystectomy or abscess irradication as indicated.

2. The passive protection tests are used to measure effects on protection of the fractionation and combination of specific Salmonella antibody components.

3. The structure of antibodies is being studied in animals previously immunized with a specific Salmonella organism. These animals are permitted to rest for several months until the specific antibodies are no longer demonstrable and then challenged with a single inoculation of a heterologous antigen. Sera drawn each day for ten days following the heterologous booster are then tested by absorption to prepare specific protective antibody components. The protection given by each antibody fraction is then estimated and compared with that obtained with a homologous booster.

4. Passive mouse immunization tests are used to measure effects on protection of the combination of specific P. proteus antibodies.

Patient Material and Major Findings:

1. Twelve Salmonella carriers are being studied. Of eight gall bladders removed from Salmonella carriers, all had the organism cultured from the center of a previously washed stone. All had received at least three weeks of antibiotic treatment prior to cholecystectomy. Liver biopsies have been sterile.

2. No immunological abnormality has been consistently demonstrated in these patients.

   Relative increases of specific antibody components in mixtures of immune sera may enhance the protective action of the serum. This has been found to be true in separate mixtures of Salmonella and of B. proteus immune sera. A paper detailing the above data is ready for publication.

3. A new antibiotic, Kanamycin, is being evaluated in these patients.

Significance to Microbiological Research:

Outbreaks of Salmonellosis represent a serious public health problem. The typhoid and other Salmonella "carrier states" are responsible for spread of the more virulent Salmonella to man, and pose a serious problem. There is no standardized therapy for the "carrier state."

Proposed Course of Project:

To continue as outlined under objectives.

With cooperation of the State Health Departments, more patients are being obtained.

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. McCullough, N. B.: Food in the epidemiology of salmonellosis. Am. Dietetic Assoc. 34: 254-257, 1958.

2. Lieberman, R., Earle, H. Ikari, N., and McCullough, N. B.: Immunity afforded by specifically absorbed, unabsorbed and combinations of Salmonella antisera. Jour. Immunol.

Honors and Awards relating to this project: None

Form No. ORP-2
November 1958

Serial No. NIAID - 19(c)
1. LCI
2. Parasitic Disease service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Helminthic Infestations.

Principal Investigator: Dr. Henry K. Beye, Dr. Donald E. Kayhoe

Other Investigators: Dr. James C. Kirby, Dr. Charles Brooks, Miss Elizabeth Guinn.

Cooperating Units: (1) Section on Chemotherapy, Laboratory of Tropical Diseases.
(2) Medical Branch, Department of State.
(3) Student Health Service, Howard University.

Man Years (calendar year 1958):
Total: 1.7
Professional: 1.3
Other: 0.4

Patient Days (calendar year 1958): 310

Project Description:

A. Filariasis (Studies concerning human filariasis, especially infections with Wuchereria bancrofti).

Objectives:

1. To clarify factors involved in host reaction and the existence of hypersensitivity in persons infected with W. bancrofti.

2. To elucidate the mechanism of action of "Hetrazan" on microfilariae and possible effects on adult worms.

3. To determine precipitating factors in the production of attacks of filarial lymphangitis.

4. To explain the mechanism of periodicity of microfilariae.

5. To determine if transmission of the disease is occurring in the United States, especially the Washington area.

6. To develop procedures and methods whereby basic studies can be done more effectively.

7. To study pathogenesis and experimental therapeutics of elephantiasis.

8. To find more effective anti-filarial drugs.

Methods Employed:

Methods whereby clinical investigations can be effectively utilized in the attainment of these objectives are administrative and technical.

Administrative aspects are chiefly related to the major problems of (1) securing suitable candidates for clinical investigation and (2) helping promote an environment whereby basic laboratory competencies are more sharply focused on human-host parasite relationships in infection and disease.

Technical aspects are concerned with the individual observations involving the collection, analysis and presentation of data concerning specific sections of the host-parasite relation.

The procurement of suitable patient candidates for this project consists of physician referrals, an arrangement with the Student Health Services, Howard University, and then very recently a cooperative arrangement with the British West Indian Labor organization. The latter mechanism is still in the process of being developed. To check the suitability of West Indian Agriculture workers as candidates, a preliminary survey of 500 persons has just been completed.

Candidates for this project will be admitted for observation and study. Basic workup consists of the routine study for admission one or two days and night study of microfilariae periodicity, x-rays of soft tissue, especially the femoral area. Additional special studies then are carried out depending upon the results and where the candidate would best fit into a protocol of special studies devised to meet the objectives as listed.

Patient Material and Major Findings:

The activities in this field were limited to just a few patients because of a number of factors: absence of the principal investigator from NIH a great part of the year, the postponement of the survey of Howard University students, and the fact that the results of the survey of the British West Indian Agricultural workers is not yet available. Major findings remain the same as those presented last year.

Significance to Microbiological Research:

The program is of considerable interest to the Institute for a number of reasons: The fact that carriers are in the United States, as well as suitable mosquito vectors which could conceivably help the infection become established within the U.S.; an estimated 186 million people in the world are infected, and the United States is committed to the expenditure of rather large sums of money in other areas in programs aimed toward the eventual control of the disease. The results described last year, particularly the prolongation of viability of microfilariae at low temperatures, open up many possibilities for more adequate study of this disease, and perhaps for fundamental host reaction studies associated with hypersensitivity. Leads on the mechanism of action of hetrazan are of great fundamental importance.

Proposed Course of the Project:

Arrangements with the Student Health Service, Howard University should continue. There is great interest among the Asian countries, including India, in filariasis, and the feasibility of NIH collaborating in necessary research before practical control measures can be applied in these countries has been under discussion. The extent and type of collaboration possible to be undertaken through NIH has not yet been determined.

If the findings of the recent survey of British West Indian laborers indicate there to be sufficient candidates, studies of new arrivals to the United States will be investigated.

Diethylcarbamazine, the present drug of choice for mass therapy, lacks much, and clinical studies can participate in helping to develop a more effective drug or to evaluate the drugs effectiveness when used with salt. Such studies will be undertaken when staff and basic laboratory knowledge becomes available.

The progress to be made in this line depends a great deal on the development of supporting technical staff, or firm collaboration with the basic laboratory workers.

B. Intestinal Nematode Infestations:

Objectives:

To correlate clinical manifestations and pathology during the various phases of the life cycle of nematodes in the mammalian host, especially man; to describe their role in the production of disease to evaluate the efficacy of established or newer antihelminthics; to investigate differences in species in terms of their clinical attributes; to improve diagnostic techniques, and to elucidate the extent and severity of infestation in the United States.

Among intestinal nematode infestations, the program embraces those species whose life cycles include a period of tissue invasion and pulmonary migrations (Ascaris lumbricoides, Strongyloides stercoralis, and hookworm) as well as those species which do not exhibit a pulmonary cycle (Enterobius vermicularis and Trichuris trichiura). Reactions of the "tissue larva migrans" type are also of interest.

Methods Employed:

Parasitic screening of State Department employees returning to the United States and of patients referred by private physicians or governmental organizations; routine stool examinations on in-patients under study in LCI, laboratory diagnostic techniques, immunological studies, and therapeutics, especially with diathazanine iodide and lucthione.

In addition, a preliminary survey for intestinal parasites was made of 140 British West Indian agricultural workers.

Patient Material & Major Findings:

1. Trichostrongylus infected patients:
Many of the State Department employees returning to the United States have been found passing eggs of this parasite in their stool. Clinical and laboratory data are lacking on this parasitic infection especially in regards to the correlation between the number of eggs per gram of stool and the possible number of parasites harbored by the host and the pathogenesis they may be producing. Furthermore, appropriate therapeutic measures against this parasite is not known. Finally, the species of Trichostrongylus observed in these patients cannot be determined until the adult worms are recovered.

2. Parasites among the British West Indies Workers:
In a recent study (November 1958) among the British West Indies, workers' blood was taken from 470 individuals to be examined for malaria and filariasis and 140 persons were examined for intestinal parasites.

To date, 100 of the slides examined have revealed NO malaria and only 1 individual in 100 has been found to be infected with filariasis.

On the other hand, 121 of the 140 (86%) workers were found to be harboring one or more types of parasites. The parasites reported were hookworm, strongyloides, ascaris, Endameba histolytica and giardia. Hookworm was the predominating parasite having a prevalence of 54% (76 of 140 stools examined). The remaining parasites had a prevalence of: 1) E. histolytica, 12%; 2) Strongyloides, 12%; 3) Ascaris, 5%; 4) Trichostrongylus, 33%; and 5) Giardia, 12%.

From these workers selected, individuals are to be brought to the Clinical Center and investigated regarding their hookworm, strongyloides and filarial infections.

Significance to Microbiological Research:

Many aspects of intestinal nematode infestations can contribute to a better understanding of host-parasite relations and lead to alleviation of disease in the United States and abroad. The finding of a relatively high prevalence of Trichostrongylus infestation among State Department employees returning from the Middle East is of significance because this infestation is rare among American as of present date. The parasitologic, clinical and epidemiologic characteristics of this infestation needs further clarification.

Within the next five years, world-wide programs against intestinal nematodes will probably be undertaken. Knowledge gained through the present programs can well be influential in modifying these programs.

Proposed Course of Project:

Present plans call for the completion of the surveys involving sewage sludge this coming year. This interesting work was reported in 1957, but we were unable to carry out further studies in 1958. Work will be intensified on investigations with Trichostrongylus, including trials of a new drug which seems effectual in the laboratory. If staff and facilities permit, fundamental studies on the mechanism of eosinophilia will be started. Patients suffering from Strongyloides and visceral larva migran infestations will provide one source of patient material for these studies.

The British West Indian workers will provide patient material for further development of this project, and closer collaboration with the section of Helminthic Diseases, Laboratory of Tropical Diseases, will enable a more intensive study of intestinal nematode infestations and the human host.

C. Trematode Infestations

1. Schistosomiasis
2. Other diseases due to flukes

Objectives:

Besides Schistosomiasis, this project is concerned with paragonimiasis and diseases due to the various liver flukes. The aim is to study systematic reactions to the infestations, immunologic responses, pathologic processes resulting from infestations, evaluation of new or experimental prophylactic or therapeutic compounds, and improvement of diagnostic techniques.

Methods Employed:

Field surveys of selected groups of people for trematode infestations, parasitic screening and workup of patients referred by private physicians or governmental organizations for suspected Trematode infestation, parasitological methods of diagnosis applied to feces, urine, bile, serologic studies, including appropriate skin tests, and reactions between ova and patient serum; clinical studies on proved cases; experimental therapy.

Patient Material & Major Findings:

Continued studies of the efficacy of Miracil D. (Miloidin), and its pharmacology indicate that it may be effective in the treatment of infestation with Schistosomiasis mansoni. The number of patients studied to date is too small to warrant definite conclusion at this time. (See patient material under "Intestinal Nematodes.")

Significance to Microbiological Research:

With the great migration of persons into the United States from areas of schistosomiasis endemicity, and the increasing number of Americans going into infested areas, plus the demonstrated increasingly larger areas of the world in which infested snails are found makes schistosomiasis a disease of increasing importance to this country as well as to the world at large.

Treatment at the present time, largely by intramuscular and intravenous routes, is costly and dangerous. In areas where the danger of reinfestation is unlikely, such as the United States, it would be advantageous to have a potent orally effective agent for the treatment of early cases of the disease to prevent the later sequelae which may be fatal.

Proposed Course of Project:

Continued efforts to evaluate new drugs with an emphasis on the pharmacological approach and thorough studies on the efficacy and toxicity of these drugs by long-term studies on persons not in endemic areas of the disease.

In association with the Section of Helminthology, Laboratory of Tropical Diseases, clinical studies will be undertaken to provide a better understanding of the pathogenesis of many of the manifestations of schistosomiasis.

D. Infestations due to Cestodes

Objectives:

To determine the extent of Cestode infestations in Americans and persons returning to the United States from other areas; to study the signs and symptoms of the disease in otherwise healthy individuals; to evaluate laboratory diagnostic procedures; and to evaluate various methods of therapy.

Taenia saginata (beef tapeworm) and Taenia solium (pork tapeworm) are of principal interest. In infestations due to these worms, studies are centered chiefly on the effects that these parasites have on the physiology of the bowel and on the effect of therapy in the erradication of the parasites.

In the diseases in which the symptoms are due to tissue invasion by the parasite, investigation is directed toward determining the effect of the parasites on various organs involved.

The dwarf tapeworms, Hymenolepis nana and H. diminuta are of interest because they are especially refractory to therapy.

Methods Employed:

Patients are secured for study through the State Department survey method and the physician patient referral system as previously described. During the past year studies on the efficacy of intraduodenal quinacrine have been under way.

Patient Material & Major Findings:

To present date, a series of fourteen patients have been treated with atabrine administered through a duodenal tube. This series is now large enough to serve as base data, for subsequent comparative studies using drugs which can be administered orally. To date, dithiazanine iodide has been tried with inconclusive results.

Significance to Microbiological Research:

Infestations with these parasites, although rare in the United States, are difficult to treat. The diseases are quite common in other parts of the world, and could potentially become a problem in this country. Cases of Echinococcus granulosa, especially among the Eskimos and Indians, present diagnostic and therapeutic problems which, because of increasing work in the study of diseases among these people, is becoming more and more appreciated. Large areas of South America are heavily infested with these parasites and in these areas they are of major importance, not only as a disease among the sheep in the areas but among the people living there. The treatment at the present time is unsatisfactory. These diseases could become established in the sheep raising areas of the United States.

Proposed Course of Project:

If time and staffing permit, surveys among sheep raising areas of this country may be of great value in determining the importance of the disease in this country at the present time.

Part B included: Yes [ ] No [X]

Form No. ORP-2
November 1958

Serial No. NIAID-20(c)
1. LCI
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Protozoal Infections

Principal Investigator: Dr. Henry K. Beye

Other Investigators: Dr. Donald E. Kayhoe (Jan. 1958 to June 1958); Dr. James C. Kirby; Dr. Charles Brooks (from July 1, 1958 to present) and Miss Elizabeth Guinn

Cooperating Units:

Section on Chemotherapy, Laboratory of Tropical Diseases, NIAID
Section on Protozoology, Laboratory of Tropical Diseases, NIAID
Medical Branch, Department of State, Washington, D. C.
Student Health Service, Howard University, Washington, D. C.

Man Years (calendar year 1958):

Total:	3.4
Professional:	1.8
Other:	1.6

Patient Days (calendar Year 1958): 125

Project Description:

A. Amebiasis

Objectives:

To improve and standardize diagnostic techniques for Amebiasis; to determine the prevalence of the parasite in persons residing in and returning to the United States; to study the possible role of strain differences, altered host resistance, associated bacterial flora and the effect of nutrition on the pathogenicity of the parasite; and to evaluate under adequate control, the efficacy of certain antiamoebic drugs.

Methods Employed:

Methods whereby clinical investigations can be effectively utilized in the attainment of these objectives are administrative and technical.

Administrative aspects are chiefly related to the major problems of (1) securing suitable candidates for clinical investigation and (2) helping promote an environment whereby basic laboratory competencies are more sharply focused on human-host parasite relationships in infection and disease.

Technical aspects are concerned with the individual observations involving the collection, analysis and presentation of data concerning specific sections of the host-parasite relation.

As has been described in previous reports, the procurement of suitable patient material for this project consists of physician referrals and a cooperative arrangement with the Medical Branch, Department of State, whereby returning State Department employees are screened for the presence of intestinal parasites. This latter arrangement has been satisfactory during 1958 as a supplementary source of patient material, especially for investigations involving Endamoeba histolytica.

During the latter part of 1958, an epidemiologic survey was conducted among West Indian agricultural workers, coming to the United States under the sponsorship of the British West Indian Labor organization, in an effort to determine the suitability of this population group for clinical studies. Preliminary results indicate that this group will be very suitable as another source of research material.

Educational activities related to amebiasis are yet another method leading to patient referrals not only by local physicians but by physicians located throughout the world.

A scientific exhibit on intestinal parasitism among State Department employees was exhibited at the "World Congress of Gastroenterology" in May 1958.

Patients who have been shown to have amebiasis are accepted for study. A history is obtained with emphasis on exposure or symptomatology that may have been a result of parasitism. A complete physical examination is done, including proctoscopy in patients with a history of diarrhea. In addition to the routine laboratory studies, special studies are done of renal and hepatic function as well as serological tests for amebiasis and other parasites. A large chest x-ray is taken on every patient, and barium enemas are done when indicated. Studies are generally done in the outpatient department unless the patient's condition warrants admission as an inpatient. Upon the completion of basic studies, the patients are placed on chemotherapy; post-treatment studies are made to determine toxicity of the drugs and their efficacy. Patients are followed when possible for one year post treatment before they are considered cured.

Patient Material and Major Findings:

The epidemiologic studies supplementary to parasitic screening of State Department employees returning to the United States and reported last year continued this year. During March and April, material concerning the first 1,453 persons surveyed was tabulated and analyzed.*

A continued compilation of this type of data for the next two to three years may provide considerable information on this infection.

In general, patients with amebiasis were found to have few major complaints, they demonstrated no abnormalities on physical examination and laboratory studies were generally within normal limits. There was only one patient with proven amebiasis whose serum was positive in the NIH complement fixation test for amoebiasis.

Patients were usually treated with chloroquine and tetracycline, and although long term studies are not completed, it would appear that the treatments were generally successful. Selected cases were treated with erythromycin, usually with a temporary flareup of complaints, but usually with eventual good results. Patients who were found to be positive after treatment with the above drugs were treated with diodiquine. There were minor complaints of gastrointestinal intolerance to the drug but most patients were able to complete the course of therapy and have had negative stools post treatment.

* This tabulation was made possible by the Medical Branch, Department of State, who supplied the clerical assistance.

In general, it might be stated that the majority of patients studied so far had mild intestinal amebiasis which responded to treatment.

Significance to Microbiological Research:

Many of the previously reported studies of amebiasis have been done in areas where adequate study was difficult on patients with conditions other than amebiasis which may have played a role in their complaints. In many areas reinfection cannot be ruled out, and consequently short term post-treatment studies have been done. On the basis of preliminary studies done in this laboratory, it would seem that the patients under study do not have the serious manifestations of the disease usually reported, nor do they seem as refractory to treatment. Because the parasite remains a potential hazard both to the patient and to his associates, we have felt that all patients harboring Endamoeba histolytica, should be treated. Little is known of the prevalence of amebiasis in the healthy American population, and practically no studies have been done on large groups returning to this country after long assignments in areas of high endemicity. Study of the data already collected among foreign service personnel would indicate that the attack rate is high in certain areas, although the amount of illness produced, even in these areas, is relatively small. These studies also indicate the inadequacies of laboratory facilities, not only in foreign areas but in the United States. With the increase in travel into certain of the endemic areas and the increase in the number of the parasites being brought into this country, the disease incidence in the United States might well be on the increase.

Proposed Course of Project:

It is felt that a good start has been made in the study of the problems of amebiasis and other parasites in this country. Continuing epidemiological studies will be necessary to adequately determine the significance of the disease in persons residing and traveling outside of the United States. The cooperative surveys underway with local universities and the State Department should be continued, since they provide much information of this nature as well as providing patients for clinical study. Efforts should be made not only to increase the number of persons studied but also to more thoroughly study each person.

It would be advantageous to expand facilities to be able to examine persons prior to as well as after foreign residence. There is a continuing need for more reliable and rapid methods of concentrating ova and cysts to improve the methods of laboratory diagnosis. It would also be most desirable to further evaluate some of the new serodiagnostic methods for amebiasis and other parasitic diseases. The method currently used seems to have its chief usefullness in the field of extraintestinal amebiasis and is not generally positive with intestinal disease alone. Efforts should be made in the futute to acquire more patients with acute amebic colitis or with extraintestinal disease. This may be possible through closer liaison with local physicians or it may be necessary to enter into field studies to study this aspect of the disease. Further studies on the role of nutrition, bacterial flora, and intercurrent diseases should be undertaken with adequate laboratory and clinical control. It is further proposed that small groups of persons engaged in foreign travel be studied to determine the efficacy of chemoprophylactic agents, and to determine more accurately the true etiology of the dysentery which frequently plagues travelers out of this country.

The clinical laboratory and treatment data on persons with E. histolytica is now being analyzed. Following the compilation of this data, the project will probably be modified and re-oriented so as to be more closely oriented with completed plans of the section of protozoology, Laboratory of Tropical Diseases.

B. Trichomoniasis

Objectives:

The objectives in the past have been to determine the extent of infection with Trichomonas and Candida among the population of females working at the National Institutes of Health; to study effects on vaginal cytology which may occur as the result of the parasite; to gain epidemiological data on the disease; and to determine the role of the parasite in the pathogenesis of certain vaginal complaints. The efficacy of various drugs was also under study.

Remarks:

During 1958, clinical investigations in this area have been sharply curtailed with emphasis being returned to the laboratory. Investigations are now underway to determine the pathogenicity of various human strains in mice. Present plans call for recommencing clinical investigations in this field after increased knowledge has been obtained in the laboratory.

C. Toxoplasmosis:

Objectives:

To study clinical and laboratory manifestations of the disease; to evaluate diagnostic methods; to study the immunologic response of the host to the parasite; and to determine effective methods of therapy in patients with proven toxoplasmosis.

Methods Employed:

Serological surveys and clinical studies on patients with symptoms suggestive of toxoplasmosis, or on patients with serological evidence of the disease, in an attempt to more clearly delineate the manifestations of the disease in its multiple forms. Recently much effort has been expended in making physicians in the area aware of the symptomatology of the disease with the expectation of this resulting in additional patient referrals.

Patient Material & Major Findings:

Continuing studies on two proved acquired cases of toxoplasmosis have given a great deal of information about the sequence of serological and skin test changes in the disease and response to combined therapy with pyramethamine and sulfadiazine. An additional patient with proved congenital toxoplasmosis has been under study; extensive studies of tissues obtained at autopsy are being performed with the cooperation of the Laboratory of Tropical Diseases and the Department of Pathologic Anatomy.

Significance to Microbiological Research:

Serologic and skin test surveys indicate that there is a high incidence of toxoplasmosis in man, yet clinical reports of the disease are relatively rare. There is much to be learned about the clinical manifestations of the disease and methods which may be employed in its diagnosis. Experience has shown that pathologic diagnosis is difficult even in patients in whom the diagnosis has been established by the recovery of the parasites by inoculation of tissues into laboratory animals. With the exception of chronic ocular toxoplasmosis, little is known of the chronic form of the disease in humans although the parasite seems to survive for long periods in laboratory animals. Studies by other investigators have suggested that the parasite may play a role in the production of myocarditis and chronic encephalitis.

Proposed Course of Project:

Continuing studies as outlined above with increased efforts to obtain clinical material for study.

D. Blood and Tissue Protozoan Disease

Objectives:

To improve diagnostic techniques; to study the clinical and pathological manifestations of these diseases in man and their pathogenesis; to evaluate various experimental therapeutic and prophylactic regimens, and to aid in the development of a chemotherapeutic agent suitable for mass therapy.

Methods Employed:

Field surveys of groups of people, as indicated; parasitic screening and work-up of patients referred as suspected cases (malaria, leishmaniasis, Chagas disease) by private physicians or governmental organizations; clinical study of proved cases; laboratory diagnostic techniques, including cytodiagnosis; immunologic studies; laboratory culture of the organisms; experimental therapy, and the utilization of human volunteers.

Patient Material & Major Findings:

The number of cases is too small to warrant definite conclusions. (See patient material under "Amebiasis").

Significance to Microbiological Research:

For the next few years emphasis in malaria will be on worldwide eradication of the parasite. Problems may develop in respect to drug reactions and resistance under the extremely variable conditions inherent in such a large program, and the development of better chemotherapeutic agents will be of great importance.

The study of these groups of diseases has significance in terms of their prevalence, and of the application of newer fundamental concepts and techniques to studies of host-parasite relation, immunological reactions, pathogenicity and therapy.

The first case of Chagas disease in the United States was reported in 1956. Inasmuch as suitable arthropod vectors for the transmission of the disease are distributed throughout most of the southwestern and western parts of the United States, this disease may assume national importance. The recent discovery of animal reservoirs for this disease in Maryland, emphasizes the necessity for greater activity in this field.

Proposed Course of Project:

The project will be greatly emphasized in 1959, primarily through its participation in the program of malaria research of the Section of Chemotherapy, Laboratory of Tropical Diseases.

Clinical investigative activities of direct concern to this project will consist first of gaining basic data on the pharmacology and anti-malarial effects of single doses of chloroquine diphosphate. Comparative studies using (1) single doses of other anti-malarial drugs, and (2) chloroquine in conjunction with a detoxifying agent or (3) chloroquine physically modified to act as a depot drug would be undertaken.

Normal human volunteers available to NIH would be utilized for the above studies. The Parasitic Disease Service would likewise participate in other studies to be conducted in the Federal Penitentiary, Atlanta, Georgia, among prisoner volunteers.

Part B included Yes [x] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Guinn, E.: The use of the zinc sulfate centrifugation flotation technique as a routine diagnostic procedure. Technical Bulletin Registry of Medical Technologists. (in press)

2. Burch, T. A., Rees, C. W., and Kayhoe, D. E.: Laboratory and clinical studies on vaginal trichomoniasis. Am. Jour. Obstetrics & Gynecology. 76: 658-665, 1958.

Form No. OEP-2
November 1958

Serial No. NIAID - 21(c)
1. LCI
2. Bacteriological Section and Pediatric Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cystic Fibrosis of the Pancreas

Principal Investigator: Dr. Norman B. McCullough (To July 1, 1958)
Dr. John P. Utz (July 1 - Present)

Other Investigators: Dr. Charles Neal and Dr. Lowell K. Good

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.3
Professional: 1.3
Other: none

Patient Days (Calendar year 1958): 965

Project Description:

Objectives:

A. To study the bacterial and fungal flora of the respiratory and intestinal tract of patients with cystic fibrosis of the pancreas, with focus upon the following points:

1) Modification of the flora by antibiotic and chemotherapeutic agents.

2) Measurement of the sensitivity of Staphlococci and of the Gram negative pathogens to various antibotics.

3) Prevention of overgrowth of pathogenic organisms and the resultant side-reactions.

4) Evaluation of the effect of nebulized antibiotics in the respiratory tract, with and without the aid of positive pressure breathing.

5) Determination of the optimal antibiotic therapy for these patients.

B. To evaluate diagnostic tests in cyctic fibrosis of the pancreas.

C. To study histochemical reactions of various tissues obtained at autopsy or by biopsy, with emphasis on the staining characteristic of the mucopolysaccharides.

D. To study the intestinal losses of albumin by the use of radioactive iodine labled polyvinylpyrrolodone.

Methods Employed:

Patients with proven cyctic fibrosis of the pancreas are employed in this study. Long-term studies of the bacterial flora of the respiratory and intestinal tracts are being made and an attempt will be made to correlate the findings with antibiotic sensitivities of the staphylococci and gram-negative rods.

A modification of the classic sweat test employing iontophoresis of pilocarpine has been evaluated. Attempts to modify the sweat electrolytes by various adrenergic and cholinergic drugs has been evaluated. Random other measurements of sweat constituents have been made.

Blood levels of various antibiotics used in treating these patients have been measured to evaluate absorption.

Studies on the histochemistry of sweat glands will be undertaken.

Patient Material and Major Findings:

At the present time, a group of approximately thirty children is being closely followed.

A. The bacteriological studies have revealed the following:

1) The bacterial flora of these patients on prolonged antibiotic therapy follows no set pattern, although the flora in those patients with severe pulmonary disease tends to have a predominance of Gram negative pathogens.

2) Of the various antibiotics used, Erythromycin systemically tended to eradicate Staphlococci most affectively.

3) Systemic antibiotic therapy is of no help in eradication of Gram negative pathogens; however, nebulization with the appropriate antibiotic was helpful in reducing the number of these bacteria and in one case in eradication of the Gram positive pathogens.

4) The appropriate antibiotic therapy as determined by sensitivity studies, and used for prolonged periods of time, definitely does benefit these patients and reduces the pulmonary symptomology.

5) Complicating fungal infections have been no problem, although minor superficial skin fungus infections are not infrequent.

B. Other studies:

1) Newer and easier methods of performing diagnostic tests for cystic fibrosis of the pancreas are being investigated. A diagnostic blood test is being sought. Investigations are under way on the amnionic fluid from mothers giving birth to children with proved cystic fibrosis of the pancreas.

2) The use of radioactive iodine labeled polyvinylpyrrolodone has shown that the amount of albumin lost via the intestinal tract is in the order of that lost by normal subjects.

Significance to Microbiological Research:

Cystic fibrosis of the pancreas is in the majority of cases a fatal disease of childhood. Simplified methods of diagnosis, improved methods of treatment and a better understanding of the basis biochemical abnormalities are needed.

Part B included Yes [ ] No [x]

Form No. ORP-2
November 1958

Serial No. NIAID-22(c)
1. LCI
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biochemical Studies (Basic and Clinical)

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Eskin Huff, Dr. Ned Feder (to August 1958)

Man Years (calendar year 1958):
Total: 4.2
Professional: 2.2
Other: 2.0

Patient Days (calendar year 1958): None

Objectives:

To study the biochemical aspects of infectious disease processes at both the cellular level and the clinical patient level.

At the cellular level, to study the biochemistry of pathogenic and antibiotic resistant organisms to learn more about the nature of antibiotic resistance.

At the clinical patient level, to develop new diagnostic procedures.

Methods Employed:

A. Basic Studies on the Nature of Antibiotic Resistance

1) Staphylococcal penicillinase

Penicillinase enzymes will be searched for in naturally occurring hospital strains of pathogenic Staphylococcus aureus. The nutritional requirements of the organism will be determined employing Warburg apparatus by measurement of the CO_2 liberated from a $NaHCO_3/CO_2$ buffer system by the penicilloic acid formed from penicillin by the action of penicillinase.

2) Cell Wall Polysaccharides

The biosynthesis of certain carbohydrates will be investigated as related to the mechanism of action of antibiotics and the development of resistance to antibiotics. An attempt will be made to determine if lactaldehyde is an intermediate in the biosynthesis of rhamnose, which occurs in the cell walls of Salmonella, Pseudomonas, Streptococcus, etc.

Major Findings:

A. Basic Studies

1) Histochemistry. A new method of preparing tissues for staining has been devised and published.

2) Viral Studies. The isoelectric point of adenovirus has been determined and studies have been published.

3) Carbohydrate Metabolism. Previous work (see Project Report NIAID-22(c), 1957) on the metabolism of 1,2-propanediol phosphate has been concluded and submitted for publication.

Work on a method for separation of acetol, lactaldehyde, pyruvic acid and methyl glyoxal by means of bisulfite ion-exchange resins has been completed and this material submitted for publication. The development of this method has made possible the positive identification of lactaldehyde as an enzymatic oxidation product of 1,2-propanediol. The enzyme has been further purified from rabbit liver and its properties studied. The results of these studies, which are almost complete (to be presented at the Federation Proceedings in April 1959), suggest that this enzyme is identical to alcohol dehydrogenase. The further metabolism of lactaldehyde is being studied at present. It is known that aldehyde dehydrogenase acts on lactaldehyde and the product of this reaction is, presumably, lactic acid. Thus, the route of metabolism of 1,2-propanediol administered to the whole animal would appear to be: 1,2-propanediol —> lactaldehyde —> lactic acid.

4) Staphylococcal Penicillinase. It has been determined that in order for some strains of S. aureus to elaborate new penicillinase in response to treatment with penicillin, the organism need not be in a growing state. For the induction of penicillinase, washed resting cells require the presence of a small number of amino acids and glucose (as an energy source). The amino acids essential for growth are also essential for the enzymic adaptation. In addition, isoleucine, an amino acid not essential for growth, is uniquely required for the elaboration of the enzyme. These findings are being prepared for presentation at the Annual Meeting of the Society of American Bacteriologists at St. Louis, Missouri, in May, 1959, and for subsequent publication.

5) Results. In the course of the work on carbohydrate metabolism, there was developed a new method for separating and characterizing aldehydes by means of bisulfite-treated ion exchange resins. While known to exist in the human body, there has been very little biochemical investigation of aldehydes per se because of the interference by glucose in most of the ordinary analytical procedures. With this new technique, it is planned to investigate the occurrence of these compounds as related to infectious disease processes.

Significance to Infectious Disease Research:

The studies in carbohydrate metabolism thus far have dealt principally with 1,2-propanediol. Although 1,2-propanediol has been used widely as a glycerol substitute in many pharmacological preparations for administration by injection or orally, the metabolism of this compound by the mammal has been little studied. The present studies have indicated that this compound is oxidized by a liver enzyme to lactaldehyde and then to lactic acid, thus explaining its glycogenic nature. The metabolism of lactaldehyde by animals and bacteria is almost unknown. Because of its chemical structure, it has been suggested to be an important intermediate in the biosynthesis of certain sugars. As outlined in the Section of Results, the role of lactaldehyde in the biosynthesis of rhamnose is now being studied.

The determination of the specific nutritional factors governing the induced formation of penicillinase by S. aureus has given us leads to the general problem of the nature of antibiotic resistance as well as a target for specific antimetabolic action. These are now being explored.

Part B included Yes [X] No [ ]

Serial No. NIAID-22(c)
1. LCI
2. Section on Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

(1) Feder, N. and Sidman, R. L.: Methods and principles of fixation by freeze-substitution. J. Biophys. & Biochem. Cytology, 4: 593-602, 1958.

(2) Steinman, H. G. and Murtaugh, P. A.: Isoelectric precipitation of adenovirus and its complement-fixing antigen. Virology, (In Press).

Honors and Awards relating to this project: None.

Form No. ORP-2
November 1958

Serial No. NIAID - 23(c)
1. LCI
2. Infectious Diseases
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Systemic Fungal Infections

Principal Investigator: Dr. Norman B. McCullough (To July 1, 1958)
Dr. John P. Utz (July 1 - Present)

Other Investigators: Dr. John P. Utz (To July 1, 1958)
Dr. Albert Treger, Dr. Med Feder
(To July 1, 1958)

Cooperating Units: Laboratory of Infectious Diseases, NIAID

Man years (calendar year 1958): Patient Days (calendar year 1958):

Total:	1.9
Professional:	1.5
Other:	0.4

1,919

Project Description:

Objectives:

1. To study diagnosis, pathogenesis and natural course of systemic fungus infections.

2. To study the problem of fungus disease due to overgrowth during antibiotic and steroid treatment.

3. To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.

4. To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.

5. To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic, and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are screened for sensitivity against a considerable number of chemotherapeutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by in vitro methods. Promising drugs are then tested in animals for acute and long term toxicity and for therapeutic effectiveness.

New methods are being developed for determining serum drug levels, rates of excretion of drug, and in vitro screening of drugs.

Patient Material and Major Findings:

1. Studies were continued on patients with a variety of culturally proved systemic fungal diseases. New patients with culturally proved disease this year included cases of cryptococcosis (2), coccidioidomycosis (2), histoplasmosis (4), blastomycosis (2), candida endocarditis or septicemia (3).

2. Work began last year on the use of a new antibiotic Amphotericin B was continued. The effect of this drug administered intravenously was evaluated in a total of 25 patients. It appears to be effective for blastomycosis and histoplasmosis. Its effectiveness in cryptococcal infections is questionable. It is of little value thus far in coccididomycosis or in severe candida infections.

3. A newer antibiotic known only as RO-2, has been shown to be very effective in fungal infections of animals and is currently being used in patients. As of December 1, 1958, three patients had been treated. Clinical studies of this drug are being done exclusively at the National Institutes of Health.

4. The laboratory continued to perform complement fixation tests for histoplasmosis implementing studies in this and other Institutes and assisting in patient recruitment. A total of 1[illegible] serums were tested during the first 11 months of 1958. Data acquired in the past years' experience with this test, when correllated with clinical studies, have allowed the conclusion that a positive titer strongly indicates active infection and disease; independent of the results of the less helpful skin test.

5. The clinical studies on the cryptococcal meningitis cases have been summarized to characterize the medical aspects of infection.

6. Attention has been called to the potentially serious nature of the occasional Aspergillus infection. Treatment of this disease has been further tested. A bizarre electrolyte disturbance associated with this treatment has been described.

7. In the course of these studies two technical improvements were made in the handling of infected animals and in preparing infected and other tissues for microscopic examination.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood; there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as monilíasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included Yes [x] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

*1. Merchant, R. K., Louria, D. B., Geisler, P. H., Edgecomb, J. H., and Utz, J. P.: Fungal Endocarditis: Review of the literature and report of three cases. Ann. Internal Med. 48: 242-266, 1958.

*2. Louria, D. B.: Some aspects of the absorption, distribution, and excretion of Amphotericin B in man. Antibiotic Med. & Clinical Therapy. 5: 295-301, 1958.

*3. Feder, M.: A method of holding mice for intravenous injection. J. Lab. & Clin. Med. 51: 662-663, 1958.

4. Utz, J. P., Louria, D. B., Feder, M., Emmons, C. W., and McCullough, N. B.: A report of clinical studies on the use of amphotericin in patients with systemic fungal diseases. Antibiotics Annual. New York, N. Y., Medical Encyclopedia, Inc., 1957-1958.

5. McCullough, N. B., Louria, D. B., Hilbish, T. F., Thomas, L. B., and Emmons, C. W.: Cryptococcosis: Clinical Staff Conference at NIH, April 25, 1957. Ann. Internal Med., 49: 642-661, 1958.

6. Utz J. P., Treger, A., McCullough N. B., and Emmons, C. W.: Amphotericin B: Intravenous use in 21 patients with systemic fungal diseases. Antibiotics Annual. 1958-1959.

7. Utz, J. P., German, J. L., Louria, D. B., Emmons, C. W., and Bartter, F. C.: Pulmonary aspergillosis with cavitation: Iodide therapy associated with an unusual electrolyte disturbance. New Engl. Jour. Med.

* Listed in last year's report as "in press."

Form No. ORP-2
November 1958

Serial No. NIAID - 24 (c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Sarcoidosis

Principal Investigator: Dr. Norman B. McCullough (to July 1, 1958)
Dr. Norman H. Bell (July 1, 1958 to present)

Other Investigators: None

Cooperating Units: Veteran's Administration, Washington, D. C.
Food & Drug Administration, Washington, D. C.
National Heart Institute
National Cancer Institute

Man Years (calendar year 1958): Patient Days (calendar year 1958):

Total:	1.0	
Professional:	1.0	1,070
Other:	None	

Project Description:

Objectives.

(1) Long-term clinical study of the course of sarcoidosis and the effect of hydrocortisone (and related steroid hormones) in altering the manifestations and course of the disease.

(2) Clinical studies attempting to demonstrate agents known to produce granulomatous disease in patients with sarcoidosis.

(3) Evaluation of the Kveim skin test as a diagnostic tool in the management of sarcoidosis.

(4) Study of the calcium metabolic abnormality and the effect of therapy in patients with this manifestation of sarcoidosis.

(5) Study of immunological mechanisms in sarcoidosis.

(6) Study of the anemia and iron metabolism in sarcoidosis.

(7) Study of vitamin D. metabolism in sarcoidosis

(8) Epidemiology of sarcoidosis.

Methods Employed:

(1) Hospitalization and outpatient observation of patients with sarcoidosis.

(2) Extensive cultural and serological study of patients with sarcoid to demonstrate etiological agents.

(3) Use of Kveim antigen and a control "antigen" preparation in patients with sarcoidosis and granulomatous diseases of known etiology. Use of pine pollen antigen in both groups of patients.

(4) Calcium balance studies on patients with sarcoidosis and hypercalcemia before, during, and after treatment

(5) Calcium infusion, renal phosphorus clearance, and phosphorus deprivation tests before and after treatment with cortisone.

(6) Tissue culture of lymph nodes removed from patients with sarcoidosis in an effort to:

a) study the characteristic aspects of growth of such tissue.

b) study cytotoxic effects of tuberculin and other antigens on such tissue.

(7) Fe^{59} iron turnover and Cr^{51} RBC survival studies.

Patient Material & Major Findings:

Approximately 50 cases of sarcoidosis have been studied here employing the above mentioned methods where applicable. Preliminary results in attempts to demonstrate known etiological agents in patients with the clinical syndrome of sarcoidosis have been unsuccessful. Calcium balance studies in progress tend to show metabolic abnormalities akin to Vitamin D intoxication in patients with hypercalciuria due to sarcoid without predictable effect of hydrocortisone on these abnormalities. Some patients behave like those with "idiopathic hypercalciuria".

We have also demonstrated that dietary intake of calcium directly affects both blood and urine levels - predominately the latter. Early results in evaluating the Kveim test indicate that it is specific and reasonably sensitive in the acute stage of the disease.

Studies also in progress have shown hypoferremia with two of six cases so studied having a rapid iron turnover. All six have had normal RBC survival as shown by Cr^{51}-tagged RBC studies.

Significance to Microbiological Research:

Sarcoidosis is a disease of unknown etiology which closely resembles granulomatous diseases of known etiology. Investigation of sarcoidosis may provide better understanding of its nature and cause and may shed light on basic immunologic phenomena of tissue allergy. Study of metabolic processes, such as calcium abnormalities, in sarcoidosis provides a basis for more precise characterization of these changes and a rational guide for experimental therapy.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included Yes ☐ No ☒

Form No. ORP-2
November 1958

Serial No. NIAID - 25(c)
1. LCI
2. Infectious Disease and Pediatric Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pyelonephritis

Principal Investigator: Dr. John P. Utz

Other Investigator: Dr. Leon G. Smith

Cooperating Units: None

Man Years (calendar year 1958):
Total: 0.9
Professional: 0.7
Other: 0.2

Patient Days (calendar year 1958): 294

Project Description:

Objectives:

1. To improve present methods of diagnosing infection.

2. To reproduce the disease in animals to delineate pathogenesis.

3. To evaluate antimicrobial drugs in patients and animals.

Methods Employed:

1. Clinical studies: After standard history, physical examination and laboratory studies newer laboratory tests will be done and treatment instituted with new drugs.

2. Animal studies: Small animals and monkeys will be used. The effects of bacterial inoculation before and after physiologic or other trauma to the kidneys will be investigated. Serial open biopsies will be done during course of infection. Biopsy material will be studied grossly, microscopically, and culturally. In the case of drugs not used before in patients, toxicity and therapeutic tests will be done on infections in animals.

Patient Material and Major Findings:

1. A number of patients have been studied clinically to define the extent of their disease. New approaches to treatment have been undertaken using hippuric acid and methionine.

2. A chemical test for nitrites has been adapted as an indirect measurement of urine for the presence of bacterial infection in the kidneys. This is based on the absence of nitrites in urine from disease-free patients and the conversion to nitrites in infected patients of normally present nitrates. A 2000 population group has been evaluated by this technique. A positive test for nitrites showed highly significant correlation with positive bacterial cultures. Furthermore, in patients presenting diagnostic problems due to abnormal urinary sediment but without bacteriuria the test was negative.

3. The technique of serial, aseptic, open surgical biopsies on small animals has been perfected. Studies have begun on the effect on the kidneys of bacteriuria due to a variety of organisms.

Significance:

"Pyelonephritis is the number one problem in infectious disease due to bacteria today." Fully 20% of autopsies show pyelonephritis and a e fifth of these cases, patients had no history or physical findings during life as warning signs of infection or disease. Furthermore, pyelonephritis is the single most common cause of secondary hypertension. New or simple tests for the presence of infection of the kidneys are urgently needed. The pathologic picture has not been, or is only with great difficulty, induced in animals and thus stages or factors in pathogenesis are poorly understood. Finally, pyelonephritis is, with the possible exception of staphylococcal infections, the most difficult bacterial disease to treat antimicrobially. Research in treatment must be continued.

Proposed Course of Study:

This project initiated formally with this report needs to be developed and pursued.

Part B Included: Yes ☐ No ☒

Narrative Report, Calendar Year 1958, for Office of the Chief, LID

During 1958, our previous Administrative Assistant, Mrs. Jeni Arliss, left to accept another appointment at NIH and was ably replaced in February by Mrs. Connie Ludden. During the year, 20 persons were separated from our employ by resignation or transfer, and 23 new personnel (including one Commissioned Officer) entered on duty. Nineteen persons, including one Commissioned Officer placed in a graded position, were promoted; and 7 other promotions are pending.

This Office has implemented the recently instituted Government Employees Training Act (PL 85-507) by supporting course work for 8 employees: all of these are taking short scientific courses out of hours at the NIH branch of the USDA Graduate School. Other educational and training activities included: continuation of detached duty of one investigator for an additional year at the Virus Laboratory of the University of California; short training periods of 4 professionals at CDC, Harvard, and Cold Spring Harbor in new techniques of fluorescent antibody applications, bacteriophages, and epidemiological methods; and one professional participated for 2 weeks in a management workshop and conference.

Out-of-ordinary contributions of this Office to the research program include arrangements (often on short notice) for volunteer studies of infectious agents and vaccines in state penal institutions; detailed contract negotiations with suppliers of tissue culture materials and viral antigens essential to major studies; and arrangement of details (including hiring of personnel) for a collaborative study with NINDB of the fetal effects of maternal viral infections.

A major problem of this Laboratory is space. Multiple fertile scientific minds investigating several problems simultaneously will invariably discover new and urgent areas for further investigation. It is often imperative to study these at once, while certain source materials are available, if the opportunity for an unique investigation is not to be lost; yet space already fully committed cannot be used. Some solution to this problem might well represent a matter for discussion at future Lab Chiefs' meetings. In addition, many of our principal investigators lack adequate office space--largely because of the inflexible design of Building #7. The space problem has been complicated by the location of a newly created Laboratory in Building 7. Changes and improvements which have been initiated or are in effect are

attempts to improve this situation. Some attic space, for example, has been converted to storage space, largely for freezers for essential virologic specimens. Air conditioning equipment, unused since conversion to a central system, is to be removed from Room 29 in Building #7, to allow for additional much-needed animal space and possibly a small serology laboratory. Two little-used laboratories are being converted to office space for the Chief, Laboratory of Bacterial Diseases; and space in the lobby has been assigned to his secretary temporarily. Partitioning of the only remaining excess corridor space, on the third floor, has made extra room for desks of some investigators. Plans for partitioning of a large chemical laboratory in Building #5 have been initiated. Some sub-zero storage space in Building #13 is being made available to us, which will permit cancellation of our present rental agreement for commercial freeze-locker space.

Communications are expected to be improved by elimination of the Building #7 central switchboard; its replacement by individual telephone extensions in each unit is in progress.

The trend to direct-fee charges for services or materials previously supplied from Central Services on a pro-rated basis, has created another new problem. We must now budget for animals and various culture media, for example; an immediate future objective is the establishment of fiscal accounting procedures in this Office for these expenditures. We propose also, and have initiated, a policy of advance planning for replacement of necessary but obsolete heavy equipment; several freezers have already been replaced.

The importance of adequate administrative and staff work in the proper functioning of a research group cannot be overestimated. The complexity of normal operation is not always appreciated by the scientist who (quite properly) demands and receives equipment, material, space and personnel essential to conduct of his research; and who, therefore, benefits by freedom from most operating detail. The administrative organization exists for no reason other than to supply this; and its successful normal operation comprises multiple daily administrative efforts which constitute continuing contributions to research programs. We feel that any accomplishment of this Office represents the aggregate of these many, small, undocumented efforts; and the published results of work in LID in 1958 indicates that we have been largely successful in maintaining the proper atmosphere for the working scientist.

NIAID
Laboratory of
Infectious Diseases

Estimated Obligations for FY 1959

Total:	$1,738,700
Direct:	1,336,400
Reimbursement:	402,300

Covering Project Reports
NIAID....50 thru 110

Serial No. NIAID-50
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Amino Acid Metabolism and Protein Turnover in Human Cell Cultures

Principal Investigator: Dr. Harry Eagle

Other Investigators: Drs. James N. Darnell, Jr., Leon Levintow, Royce Z. Lockart, Jr., Mr. Ralph Fleischman, Mr. Aaron Freeman, Miss Mina Levy, and Mr. Vance I. Oyama.

Cooperating Units: NIDR, Dr. Karl A. Piez
NCI, Dr. Edward L. Kuff

Man Years (calendar year 1958):
Total 8 7/12
Professional: 5 2/12
Other: 3 5/12

Project Description:

A. Objectives: Biosynthesis and utilization of "non-essential" amino acids by human cells in culture.

Methods Employed: The techniques employed in the culture of these cells, in the separation and identification of the amino acids and their metabolic products, and the use of isotopically labeled precursors have been described in detail in previous publications relating to this project.

Major Findings: 1. Serine is one of 7 amino acids (alanine, asparagine, aspartic, glycine, glutamic, proline, serine) which are nutritionally non-essential for mammalian cells in culture, in the sense that they are made from other essential components of the medium, specifically, glucose and glutamine. Serine has, however, proved essential for the growth of single human cells. The same basal medium which, supplemented with serum protein, permitted the indefinite propagation of large cell inocula failed to support the growth of single cells unless serine were added. Studies are in progress to determine the factors underlying this serine requirement.

Part B included.

2. Cystine was originally considered to be an essential amino acid in cell cultures, in that it could not be formed from methionine. More recent studies have however shown that most cell cultures can grow in a cystine-free medium, provided that the cells are supplied with reduced inorganic sulfur, or compounds which can serve as a source of sulfur. D-cystine, D- or L-homocyst(e)ine, cyst(e)amine, and thioglycollate have all proved effective. The mechanism of cystine biosynthesis is under continuing study. Surprisingly, the inorganic sulfur is not used directly; and glucose accounts for less than half of the carbon skeleton of the cyst(e)ine formed.

Significance to the Program of the Institute: Studies on the nutritional requirements of human cells, their metabolism, and biosynthetic processes are of basic significance in relation to any biological studies involving cells or tissues. For NIAID research specifically, studies of the kind here described are relevant to the problems of antibody production, sensitization, phagocytosis, inflammation, virus propagation, and many other problems which are the concern of the microbiologist interested in the study of human disease. The fact that these studies are equally important to other disease areas (e.g. cancer, heart disease, etc.) does not lessen their significance for NIAID.

Proposed Course of Project: Studies will be continued on the mechanism of both these phenomena.

B. The amino acid pool of cultured cells and its significance for protein synthesis (Eagle, Piez, Fleischman).

Objectives: To determine the degree to which individual amino acids are concentrated by a variety of human cells.

Methods Employed: See under A.

Major Findings: Amino acids have been found to be concentrated by human cells in culture. The ratio of intracellular: extracellular amino acid varies inversely with its concentration in the environment, from approximately 25-fold at 0.00 1 mM, to 3-fold at 10 mM. Only minor differences have been found between individual amino acids, and no differences between various cell lines. The kinetics of this equilibration are under investigation. It has further been shown that dipeptides are transported as such into the cell, and there hydrolyzed to the component amino acids.

Cells grown in tissue culture on a medium deficient in vitamin B6 do not give maximal yields of poliovirus unless a supplement of nutritionally non-essential amino acids is provided. This finding has been correlated with the absence of serine, alanine, glycine and proline from the soluble amino acid pool, suggesting a functional impairment of the enzymatic reactions leading to the synthesis of these amino acids. The glutamic-oxalacetic transaminase, leading to the synthesis of aspartic acid, is many times more active than the other transaminases, probably explaining the persistence of aspartic acid in the pools of the B6-deficient cells. The absence of proline suggests a hitherto

unsuspected role of vitamin B6 in the biosynthesis of this amino acid. In contrast to the foregoing findings, the pool of essential amino acids in the B6-deficient cells is normal, implying that the transport and concentrating mechanisms of the cells are not impaired by this degree of vitamin B6 deficiency.

When the cells were supplied with a medium deficient in the essential amino acids, the missing amino acids were depleted from the pool within several hours, but the non-essential amino acids were retained at normal or augmented levels for prolonged periods. This observation indicates that the cell retains its capacity to synthesize and maintain pools of non-essential amino acids even under conditions of acute deficiency of essential amino acids.

Significance to the Program of the Institute: See under A.

Proposed Course of Project: Studies are in progress to determine the amino acid concentration in the environment necessary for the initiation of cell growth, and thus, the magnitude of the cell amino acid pool necessary for protein synthesis. Concurrently, attempts are in progress to determine the magnitude of the cell pool necessary for viral synthesis.

C. Protein turnover in human cell cultures (Eagle, Piez, Fleischman).

Objectives: To establish whether cellular proteins are continuingly being broken down and resynthesized, or whether, as in bacterial cultures, protein once made is relatively stable.

Methods Employed: See under A.

Major Findings: Studies have been completed which clearly indicate that, unlike bacteria, there is a continuing process of protein turnover in mammalian cell cultures. This protein renewal, evidenced by the incorporation of labeled amino acid residues into protein, takes place at the surprising rate of 1% per hour, and occurs at the same rate in both growing and non-growing cultures. Teleologically, this may be a mechanism whereby the cell can replace non-functioning enzymes.

Significance to the Program of the Institute: See under A.

Proposed Course of Project: Studies are in progress to determine whether the turnover process affects all of the cell proteins, or only a fraction, and whether individual proteins turn over at the same rate. The relative rate of turnover in structural elements of the cell (nucleus, mitochondria, microsomes) is also under study.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Salzman, N.P., Eagle, H., and Sebring, E.D. The Utilization of Glutamine, Glutamic Acid, and Ammonia for the Biosynthesis of Nucleic Acid Bases in Mammalian Cell Cultures. J. Biol. Chem., 230: 1001-1012, 1958.

Eagle, H., Freeman, A.E., and Levy, M. The Amino Acid Requirements of Monkey Kidney Cells in First Culture Passage. J. Exp. Med., 107: 643-652, 1958.

Piez, K.A., and Eagle, H. The Free Amino Acid Pool of Cultured Human Cells. J. Biol. Chem., 231: 533-545, 1958.

Eagle, H., Barban, S., Levy, M., and Schulze, H.O. The Utilization of Carbohydrates by Human Cell Cultures. J. Biol. Chem., 233: 551-558, 1958.

Eagle, H., and Foley, G.E. Cytotoxicity in Human Cell Cultures as a Primary Screen for the Detection of Anti-Tumor Agents. Cancer Research, 18: 1017-1025, 1958.

Foley, G.E., and Eagle, H. The Cytotoxicity of Anti-Tumor Agents for Normal Human and Animal Cells in First Tissue Culture Passage. Cancer Research, 18: 1012-1016, 1958.

Eagle, H., Piez, K.A., Fleischman, R., and Oyama, V.I. Protein Turnover in Mammalian Cell Cultures. J. Biol. Chem. In press.

Lockart, R.Z., Jr., and Eagle, H. Requirements for Growth of Single Human Cells. Science. In press.

Eagle, H. Amino Acid Metabolism in Human Cell Cultures. Science. In press.

Honors and Awards relating to this project:

Jan. 1958-Jan. 1959 - President, Society of American Bacteriologists.

10-1-58 - Appointed member, Cell Biology Study Section, Division of Research Grants, NIH.

11-15-58 - Appointed member, Board of Governors, American Academy of Microbiology.

Lectures:

2-23-58 - Seminar, Perspectives in Virology, New York City.

3-4-58 - Tritt Memorial Lecture, Washington University School of Medicine.

Part B. continued

3-12-58 - Symposium, Cancer Chemotherapy Screening Procedures.

3-24-58 - Symposium, Chemical Basis of Development, Johns Hopkins University.

5-19-58 - Lecture, Ohio State University.

6-16-58 - Gordon Research Conference, New Hampton School, New Hampton, New Hampshire.

8-1-58 - VII International Congress for Microbiology, Stockholm, Sweden.

9-2-58 - IV Congress of Biochemistry, Vienna, Austria.

11-13-58 - Seminar, University of Minnesota Medical School.

Serial No. NIAID 51
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Transamination Reactions with Peptides.

Principal Investigator: Dr. Stanley Barban

Other Investigators: Mr. Henry O. Schulze

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1-3/12
Professional: 6/12
Other: 9/12

Project Description:

Objectives: 1. To study how peptides are utilized in tissue culture.

Methods Employed: The techniques employed in the culture of the cells have been described in detail in previous publications relating to this project. Cell-free extracts were prepared by sonic disruption; identification of amino acids and their metabolic products were performed by chromatographic, spectrophotometric, and various enzymatic techniques. Enzyme fractionation was done by differential centrifugation, salt fractionation, and absorption and elution from gels.

Major Findings: It has been previously found that certain peptides could support growth of tissue cells in place of their corresponding essential amino acids. Cell-free extracts have been shown to use peptides as amino donors in transaminating reactions. Since these extracts also contain peptidases, the mechanism of the transaminates was unknown; i.e., whether the peptides were split prior to transamination or after. By means of various enzyme fractionation procedures, it has been possible to separate the peptidase from the transaminase enzymes. Preliminary evidence suggests that peptides may be amino donors in transamination reactions.

Part B Included

Significance to the Program of the Institute: To date, there has been no evidence for the enzymatic role of peptides in transamination. The reversal of this reaction would lead to the formation of peptides. An understanding of the mechanism of peptide formation is of basic significance to biological problems involving growth of cells and tissues. For NIAID research specifically, these studies are relevant to the problems of virus propagation, antibody formation, sensitization, phagocytosis inflammation and other related biological phenomena.

Proposed Course of Project:

1. To further purify the enzyme fractions.

2. To study the precise mechanism of the involvement of peptides in transamination.

3. To study the reverse of this reaction.

4. To determine the specificity of this reaction with various di- and tri-peptides.

Serial No. NIAID 51

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Barban, S., and Schulze, H.O., Transamination Reactions in Mammalian Cells in Tissue Culture. J. Biol. Chem. In press.

Honors and Awards relating to this project: None

Serial No. NIAID 52
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Carbohydrate Metabolism in Cell Culture.

Principal Investigator: Dr. Stanley Barban

Other Investigators: Mr. Henry O. Schulze

Cooperating Units: None

Man Years (calendar year 1958)
Total: 1-4/12
Professional: 6/12
Other: 10/12

Project Description:

Objectives: To study the metabolic pathways by which certain carbohydrates are utilized by human cells in culture.

Methods Employed: The techniques employed in the culture of these cells, in the separation and identification of the amino acids and their metabolic products, and the use of isotopically labeled precursors, have been described in detail in previous publications relating to this project.

Major Findings: It had previously been found, that glucose metabolism by a number of cell lines is characterized by a very active phosphorylating mechanism and high aerobic glycolysis, while galactose is metabolized in the absence of measurable phosphorylating activity on aerobic glycolysis suggesting an altered pathway for

Part B Included

galactose utilization. Recent studies with isotopically labeled galactose indicate that extracts of these cells do possess a very weak hexokinase for phosphorylating galactose. Further enzymatic studies implicate the uridine nucleotides in the subsequent metabolism of galactose phosphate.

Significance to the Program of the Institute: An understanding of the metabolic pathways involved in carbohydrate utilization has broad implications, e.g., energy relationships in growth, mechanism of virus propagation, antibody formation, sensitization, inflammation, nucleic acid formation and other problems of concern to the microbiologist interested in the study of human disease. These studies are also intimately related to other disease processes, e.g., cancer, heart disease, and other malignancies.

Proposed Course of Project:

1. To further elucidate the pathway by which galactose is metabolized.

2. To determine fundamental differences, if any, between glucose and galactose metabolism.

3. To study the effect of various metabolic inhibitors on carbohydrate metabolism.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Eagle, H., Barban, S., Levy, M., and Schulze, H. O., The Utilization of Carbohydrates by Human Cell Cultures. J. Biol. Chem. 233: 551-558, 1958.

Honors and Awards relating to this project: None

Serial No. NIAID 53
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Genetic Studies with Cultivated Human Cells

Principal Investigator: Dr. Robert I. DeMars

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2 6/12
Professional: 1
Other: 1 6/12

Project Description:

Objectives:

1. To find and describe genetic variations (mutations) in cultivated human cells.

2. Suitable types of mutations will then be adopted as standards for the development of useful mutagenic techniques.

3. With the aid of techniques developed (1) and (2) we aim to develop cell strains having numerous genetic alterations. Strains with suitable arrays of such genetic 'markers' will be used in attempts to transfer genetic material from one cell to another.

Methods Employed: The cell cultivation techniques are those in general use in this laboratory. The general approach is to treat a culture of human cells as the microbial geneticist treats cultures of bacteria or viruses.

Part B included.

Results:

1. Thymine starvation and selection for auxotrophic mutants in cultures of human cells. Cells that, through mutation, lose a synthetic capacity are termed auxotrophs. Such cells have at least one more nutritional requirement than does the 'wild' type cell. We have found with HeLa cells, that thymine starvation induced by aminopterin or by 5- Fluorodeoxyuridine, leads to cell death, i.e., loss of ability to proliferate. However, where cells are unable to grow due to a nutritional insufficiency of the medium, thymine starvation fails to kill and the cells can be recovered by removal of the selective agent and provision of the missing nutritional factors. This situation permits effective selection for auxotrophs that cannot grow in the basal tissue culture medium. It has now been used to isolate rarely occurring glutamine-requiring cells from large populations of cells that do not require glutamine, and is being applied in a search for other types of auxotrophs.

2. Mutation to aminopterin resistance. Rarely occurring cells in the HeLa population can proliferate in the presence of aminopterin. Different clonal populations of resistant cells exhibit characteristically different, genetically determined levels of resistance. There are at least three different levels. At present we know nothing about the relation between the mutations leading to different levels or about the basis of resistance to aminopterin.

3. Reversions to prototrophy. Some strains of HeLa cells are unable to grow in media containing glutamic acid in place of glutamine. In one of these glutamine-requiring populations, S3R1, mutations to glutamine independence occur. Such prototrophic mutant constitute about 10^{-5} of the population. We have found that the mutations concerning aminopterin resistance, glutamine requirement and poliovirus semi-resistance (Darnell) occur independently of one another and, therefore, almost certainly reside in different regions of the genetic material of these cells.

Significance to the Program of the Institute:

1. The study of auxotrophic mutants should increase our understanding of the nutrition of human cells.

2. We have pointed up three important aspects of the mode of action of aminopterin:

(a) It institutes a thymine deficiency that can lead to cell death.

(b) Thymine deficiency fails to kill when cell synthetic processes are blocked due to a nutritional or other deficiency.

(c) Human cells can undergo genetic changes leading to aminopterin resistance.

Such information may prove useful in understanding the limitations of aminopterin as a cancer chemotherapeutic agent and in developing more effective treatments.

3. Our major objective is the development of a set of basic tools for studying human genetics in vitro. The importance of such knowledge is evident from the increasing prominence of genetics in modern medicine.

Proposed Course of Project: The method of thymine starvation is being used to obtain as many auxotrophic mutants as possible.

The mutations to aminopterin resistance and to glutamine independence constitute useful systems for developing mutagenic techniques. We are now testing azaserine, triethylene melamine and X-rays as mutagenic agents for these cells.

At present we have two HeLa cell strains that differ in three genetic respects. We have started attempts to transfer genetic material between these strains.

Serial No. NIAID 53

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

DeMars, R. I. Selection for Auxotrophic Mutants in Cultures of Human Cells - manuscript in preparation.

Honors and Awards relating to this project: None

Serial No. NIAID 54
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Mechanisms of Virus Production in Tissue Culture

Principal Investigator: Dr. James E. Darnell, Jr.

Other Investigators: Drs. Harry Eagle, Leon Levintow, and Mr. Thomas K. Sawyer

Cooperating Units: None

Man Years (calendar year 1958):
Total: 4 2/12
Professional: 2
Other: 2 2/12

Project Description:

Objectives:

1. Nutritional requirements for poliovirus replication in HeLa cells, with particular reference to qualitative and quantitative amino acid requirements.

2. Purification and chemical analysis of poliovirus and use of purification techniques to determine origin of viral constituents.

3. Variation in poliovirus susceptibility among HeLa cell strains.

4. Effect of Neutral Red on plaque formation in animal cells.

Methods Employed: Methods of cell culture and virus titration have been published (Adsorption and Maturation of Poliovirus in Singly and Multiply Infected HeLa Cells, J. Exp. Med. 107: 633-641, 1958. Glucose and Glutamine in Poliovirus Production, Virology 6: 556-566, 1958). New techniques, including growth and infection of suspension cultures of mammalian cells, cellulose column purification of poliovirus, and the clonal isolation of mammalian cells, have been adapted to our needs.

Part B included.

Major Findings:

1. Previous published work indicated the importance of glucose and glutamine in the medium in poliovirus synthesis, but gave no clue as to the possible source(s) of other constituents of virus. In experiments with suspension cultures under conditions where the free amino acid level in the cell pool is low, virus synthesis has been shown to be dependent on the addition of essential amino acids to the medium. The conclusion seems justified that cell protein is unavailable for direct conversion to virus material.

2. Simple techniques for the concentration and purification of poliovirus have yielded virus preparations containing no demonstrable cellular contaminants. Relatively large amounts of virus can be conveniently prepared from large volumes of suspension cultures.

3. Clonal strains of HeLa cells have been isolated which differ widely in their relative susceptibilities to poliovirus. Analysis of the differences in these cell lines reveals that adsorption of virus is equivalent, and that the difference in susceptibility is independent of virus source. It appears that this difference in susceptibility is due to a genetic difference in the cell lines.

4. It has been shown with two different cell virus complexes that neutral red incorporated into an agar overlay decreases the number of plaques which can be obtained. This is of practical importance, since neutral red is widely used to demonstrate a virus-killed area of cells in plaque assays.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Darnell, J.E., Jr. Adsorption and Maturation of Poliovirus in Singly and Multiply Infected HeLa Cells. J. Exp. Med., 107: 633-641, 1958.

Darnell, J.E., Jr., and Eagle, H. Glucose and Glutamine in Poliovirus Synthesis. Virology, 6: 556-566, 1958.

Darnell, J.E., Jr., Lockart, R.Z., Jr., and Sawyer, T.K. The Effect of Neutral Red on Plaque Formation in Two Virus-Cell Systems. Virology, 6: 567-568, 1958.

Serial No. NIAID 55
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of a Genetics of Mammalian Cells in Culture.

Principal Investigator: Dr. Leonard A. Herzenberg

Other Investigators: None

Cooperating Units: Leukemia Studies Section, NCI (Dr. Robert Roosa)

Man Years (calendar year 1958):
Total: 1-5/12
Professional: 1
Other: 5/12

Proj Project Description:

Objectives:

1. To establish chemically defined cultural conditions for growth in culture of ascites tumor cells of inbred lines of mice.

2. To develop methods for analyzing the genetic characteristics of these cells including *in vitro* (cell culture) and *in vivo* (ascites) techniques.

3. To select variant lines of these cells having identifiable, heritable characteristics which can be used as genetic markers in attempts at finding interchange of genetic material between these cells.

Methods Employed: The basic techniques and media of mammalian cell culture developed and employed by Dr. Harry Eagle and his group served as the methodological basis for this project.

Part B Not Included

Major Findings:

1. New growth factor requirements. A histiocytic neoplasm (leukemia) derived from a DBA/2 mouse and established as a continuous cell culture (P 388 (Dawe)) was found to grow optimally in Eagle's basal medium (BME) supplemented with 5% whole serum, but not to grow at all in BME supplemented with any concentration of dialyzed serum. Full growth was, however, obtained in this latter medium supplemented with 0.2mM serine or glycine, or 1mM pyruvate, glycolate, alpha keto butyrate or alpha keto n-caproate. An increased but not maximum rate of growth was obtained with 1mM alanine. No other amino acids, Kreb's cycle intermediates, fatty acids, co-factors, or other substances tried supported growth.

Through the use of radioactive glucose or pyruvate, it was established that serine, glycine and alanine in the cellular protein do not arise from exogenous pyruvate, and that the effective supplements therefore act in some way other than as simple precursors for these amino acids.

2. With the development of an adequate defined medium it became possible to grow populations of P 388 from single cells by simply allowing a few cells to develop in a Petri dish and picking up the resultant clonal populations which arise in the form of a colony on the glass. Injections of these clonal stocks into mice of suitable inbred lines results in ascites tumor development.

3. Selection of drug resistant variants. Variant lines of P 388 were obtained by selection in the presence of gradually increasing concentrations of amethopterin which were resistant to this drug. Several distinct lines were obtained which were able to grow in different concentrations of the drug. The resistance is heritable and retained during growth in the absence of drug for periods of at least 90 generations. Gross resistance to another anti-folic acid drug, aminopterin was demonstrated. Reconstruction experiments showed that single drug resistant cells could be routinely isolated from a population consisting of resistant and sensitive cells in a ratio of 1:100,000.

Significance to the Program of the Institute: Studies of the fundamental biological properties of mammalian cells are of utmost importance to an understanding of the normal and diseased functioning of the organisms made up of these cells.

Proposed Course of Project:

1. To achieve an understanding of the biochemical basis of the nutritional requirement for certain alpha keto acids by P 388.

2. To look for the generality of the above.

3. To determine the genetic parameters of drug resistance in cell culture.

(a) To determine if resistance is due to spontaneous mutation followed by selection or is due to a "directed" change.

(b) To determine if resistance develops in a truly stepwise fashion.

(c) To determine the frequency of occurrence of resistant cells.

(d) To attempt to modify this frequency.

4. To attempt to find a transfer of genetic material (information) between suitably marked lines.

Serial No. NIAID 56
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Nucleic Acid Biosynthesis in Cell Cultures

Principal Investigator: Dr. Norman P. Salzman

Other Investigators: Mr. Edwin Sebring

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1-8/12
Professional: 8/12
Other: 1

Project Description:

Objectives: To elucidate the pathways by which ribonucleic acid (RNA) and deoxynucleic acid (DNA) are synthesized by human cells in tissue culture, and the effect of viral infection on nucleic acid biosynthesis.

Methods Employed: The techniques employed in the culture of these cells, in the separation and identification of the amino acids and their metabolic products, and the use of isotopically labeled precursors have been described in detail in previous publications relating to this project. The purine and pyrimidine bases have been isolated by appropriate chromatographic procedures and the RNA and DNA separately isolated and analyzed.

Major Findings:

1. Fluctuations in RNA, DNA and protein under varying conditions. Cultures grown adherent to glass showed wide but systematic fluctuations in the cellular contents of RNA, DNA and protein depending on the age of

Part B Included

the culture. The lag phase is characterized by a rapid synthesis of RNA, DNA, protein and acid-soluble nucleotides, at a rate which exceeds that of cells in the logarithmic phase of growth. The levels of protein, RNA, and DNA attained exceed those found in rapidly proliferating cells, and are then reduced sharply when growth commences. The levels of RNA and protein continue to decline during the logarithmic and the stationary phase, and without relationship to the rate of cellular division. In contrast, the excess DNA which accumulates during the lag phase disappears from the cell by the second or third day, and the DNA per cell thereafter remains relatively constant.

Suspension cultures maintained in the log phase of growth undergo balanced growth with the cellular RNA, DNA, and protein remaining constant. This behavior of suspension cultures makes it more suitable for certain metabolic studies (see project on poliovirus).

2. Studies on the exchange of materials between the polymeric RNA and DNA and the small molecules in the acid soluble fraction of the cell (turnover) using prelabeled log phase and non-growing cultures has demonstrated that in growing cultures RNA does turn over, and that the products of RNA breakdown can be utilized for the synthesis of DNA. Turnover would appear to be more rapid under non-growing conditions since almost as high a level of incorporation into RNA is observed in a non-growing as in a growing culture.

3. The incorporation of several precursors into the nucleic acids of growing mammalian cell cultures has been determined. Adenine is effectively incorporated into nucleic acid adenine and guanine, while guanine is less efficiently utilized as a source of RNA and DNA guanine and is converted to adenine to only a limited degree. The limited incorporation of guanine is the result of its rapid catabolism to xanthine, rather than an inability of the cell culture to utilize the free base.

In addition to its ability to utilize preformed purines the HeLa cell can synthesize purine bases de novo, as measured by the efficient utilization of glycine for purine biosynthesis.

Of the precursors which were incorporated only into the nucleic acid pyrimidines, cytidine was effectively used as a source of cytosine, uracil and thymine, while orotic acid, which also labeled these three bases, was poorly utilized. Thymine and thymidine both were incorporated solely in DNA thymine, though only thymidine was used effectively. The various purine and pyrimidine precursors studied are used by the HeLa cell in a manner qualitatively similar to that observed in in vivo animal studies.

Cytidine-2-C^{14} and orotic acid-6-C^{14} are more efficiently utilized for the synthesis of RNA uracil than for RNA cytosine. This difference is not due to dilution of the precursor by pre-existing cell pools, since the uracil pool is several fold higher than the pool of cytosine compounds. The preferential incorporation of either cytidine or orotic acid into RNA uracil may instead reflect a rapid turnover of a small fraction of the RNA significantly richer in uracil than the rest of the cell RNA.

Significance to the Program of the Institute: The studies which elucidate the metabolic behavior of the nucleic acids in uninfected cultures are needed in order to interpret the effects seen after virus infection.

Proposed Course of Project:

1. Additional studies of turnover under non-growing conditions will be carried out.

2. The studies on the fluctuations of RNA, DNA, and protein have been completed.

3. The elucidation of pathways of synthesis of the nucleic acid will be extended as it relates to the virus study.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salzman, N.P., Systematic Fluctuations in Cellular Protein RNA and DNA during Growth of Mammalian Cell Cultures. Biochem. Biophys. Acta, in press.

Honors and Awards relating to this project: None

Serial No. NIAID 57
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Alterations in Cell Metabolism Resulting from Poliomyelitis Infection.

Principal Investigators: Drs. Norman P. Salzman, and Royce Z. Lockart, Jr.

Other Investigator: Mr. Edwin D. Sebring

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1 4/12
Professional: 6/12
Other: 10/12

Project Description:

Objectives: To determine the alterations in cell metabolism resulting from infection with poliomyelitis virus, and the primary site of action of the virus.

Methods Employed: The techniques employed are those involving radioactive precursors, the separation of the nucleic acids and the isolation of the purified purine and pyrimidine bases, and methods for measuring poliovirus using the plaque assay.

Major Findings: Log phase suspension cultures of HeLa cells have been infected with poliovirus at a multiplicity which results in simultaneous infection of all cells. An inhibition of net synthesis of RNA, DNA and protein is observed. Six to twelve hours after addition of the virus there is a sharp loss of RNA from the cell.

Part B not included.

The loss of 40 per cent of the cellular RNA at a time when there is no loss of protein suggests that this loss is not due to generalized cellular damage. An enhanced nucleotide pool in virus infected cells has also been observed.

Similar patterns of cytidine-2-C^{14} in uptake are observed in non-growing and polio-infected cultures, differing from the pattern of incorporation by growing cultures.

The loss of cellular RNA, and the enhanced nucleotide pool are observed only in the virus-infected culture.

Significance to the Program of the Institute: The poliomyelitis-HeLa cell system may serve as a model system for an RNA-containing virus which has profound cytopathogenic effects. It is possible that various inhibitors may alter the course of infection. An understanding of the primary site of virus action would be of great value.

Proposed Course of Project: Methods for the separation of polymeric RNA into various species are being devised, and cells will be examined to determine whether polio infection results in the formation of a unique RNA.

Experiments designed to elucidate the primary site of virus infection will be continued.

Serial No. NIAID 58
1. Infectious Diseases
2. Experimental Therapeutics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Use of Tissue Culture Techniques to Study Basic Virus-Cell Interaction.

Principal Investigator: Dr. Royce Z. Lockart, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 12/12
Professional: 8/12
Other: 4/12

Project Description:

Objectives: To carry out a quantitative study of the virus-cell relationships in the phenomenon of viral interference primarily to gain insight on the effects of viruses on cells and their possible implications in the formation of carrier cultures.

Methods Employed: Methods used have been published (Some Factors Influencing the Interaction of Western Equine Encephalomyelitis Virus and Selected Host Cells. J. Inf. Dis. 103: 163 (1958)).

Major Findings: Previous work showed that Western Equine Virus inactivated at 37° can protect cells from the cytopathogenic effects of fully active homologous virus and greatly reduce their yield. It is also known that Western Equine virus can be carried indefinitely

Part B Included.

in L cell cultures which give all appearances of normal healthy cultures. Recent work strongly indicates that inactive virus (interference) is responsible for the formation of carrier cultures; and recent experiments suggest that cytopathogenic effects can be inhibited even in the presence of continued virus production.

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Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Darnell, J.E., Jr., Lockart, R.Z., Jr., and Sawyer, T.K. The Effect of Neutral Red on Plaque Formation in Two Virus-Cell Systems. Virology 6: 567-568, 1958.

Honors and Awards relating to this project: None

Serial No. NIAID 59
1. Infectious Diseases
2. Medical & Physiologic Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Reversal of Antibiotic Activity of Aureomycin by Culture Filtrates of Pseudomonas Species.

Principal Investigator: Dr. Arthur K. Saz

Other investigator: Mrs. L. Marina Martinez
Miss Elisabeth Y. Pilgren

Cooperating Units: None

Man Years (calendar year 1958)

Total:	21/12
Professional:	6/12
Other:	15/12

Project Description:

Objectives: To investigate the anti-Aureomycin activity of culture filtrate of various Pseudomonas species. To purify, isolate and identify compound(s). To determine relationship, if any, to mode of action of Aureomycin.

Methods Employed: Standard bacteriological and such biochemical techniques as may be indicated.

Major Findings: Culture filtrates of various Pseudomonas species possess the capacity to reverse the antibiotic activity of Aureomycin vs. E. coli and Staph aureus. Various considerations indicated that the reversal was not due to an Aureomycin-destroying enzyme but was rather due to an organic molecule possessing the ability to reverse the activity The factor is alkali-stable, heat-stable, acid-labile, dialyzable and not precipitable by alcohol or acetone. It has been concentrated from dialysates. It has not been found in E. coli or Proteus species filtrates.

Part B included: Yes [ ] No [x]

Significance to the Program of the Institute: Insofar as the factor may be of value in helping to define the mode of action of an antibiotic, it is of value to an organization interested in various aspects of the treatment of infectious diseases. Further, identification of the factor may aid in explaining naturally occurring resistance of various organisms to Aureomycin.

Proposed Course of Project: To attempt to purify and isolate the factor by means of paper and column chromatography and electrophoresis. To determine whether other tetracyclines, i.e., terramycin and achromycin, are reversed by this factor.

Serial No. NIAID 60
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Mode of Action of Aureomycin and Biochemical Mechanism of Resistance Thereto.

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: Mrs. L. Marina Martinez
Miss Elisabeth Y. Pilgren

Cooperating Units: None

Man Years (calendar year 1958)
Total: 12/12
Professional: 3/12
Other: 9/12

Project Description:

Objectives: To determine the biochemical basis for the inhibition of sensitive bacteria by Aureomycin, and the biochemical mechanism of resistance of bacteria to the antibiotic. To study methods of increasing the sensitivity of Aureomycin-resistant bacteria to the antibiotic.

Methods Employed: Isolation, resolution and purification of various electron transport enzymes from both sensitive and resistant E. coli will be carried out.

Major Findings: It has been found that various electron transport cell-free enzyme systems derived from Aureomycin-sensitive E. coli are strongly inhibited by the antibiotic. On the other hand, similar systems derived from Aureomycin-resistant E. coli (secured from the parent, sensitive strain by serial passage in antibiotic containing media) are resistant to the antibiotic.

Further properties of the factor present in serum albumin which stimulates the reduction of 2, 6 dichlorophenol-indophenol by E. coli extract and potentiates the inhibitory activity of Aureomycin on these reductions have been determined. It is heat labile, alkali labile, is not replaceable by any of a variety of free metals and is non-dialyzable. it is not any of a variety of vitamins or conjugates of vitamins.

Part B included: Yes [X] No [ ]

Significance to the Program of the Institute: On an applied level, studies on the mode of action of antibiotics, and on the mechanisms involved in the development of resistance to the compounds provide a rational approach to the chemotherapy of infectious diseases.

In addition, understanding of the mechanisms involved in increasing the sensitivity of resistant organisms to an antibiotic is of obvious clinical importance.

On a basic level, an understanding of the main energy-yielding processes of the cells (electron transport) and the significance of both stimulation and inhibition of these enzymes is of great theoretical significance.

Proposed Course of Project: It is proposed to continue studying the electron transport systems of sensitive and resistant cells and to purify and isolate the factor(s) from albumin which both stimulate the dye reduction and potentiate Aureomycin activity. Column and paper electrophoresis will be used in these attempts.

Serial No. NIAID 60

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Saz, Arthur K. and Martinez, L. Marina: Enzymatic basis of resistance to Aureomycin. II. Inhibition of electron transport in Escherichia coli by aureomycin. J. Biol. Chem. 233: 1020-1022, 1958.

Serial No. NIAID 61
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Autolysis in Genus Bacillus. Mechanism of Cysteamine-induced Lysis in the Genus Bacillus.

Principal Investigator: Dr. Arthur K. Saz

Other Investigator: None

Cooperating Units: Dept. of Bacteriology
Indiana University
Bloomington, Indiana
(Dr. Eugene D. Weinberg)

Man Years (calendar year 1958)
Total: 12/12
Professional: 3/12
Other: 9/12

Project Description:

Objectives: A study of the biochemical mechanism of cysteamine-induced lysis of Bacillus subtilis.

Methods Employed: Making of cell-free extract by various means, paper and column chromatography and electrophoresis and other methods as may be indicated by results obtained. Standard bacteriological techniques.

Major Findings: Several other compounds, most either related to cysteamine, or potentially able to be metabolized to cysteamine, have been found which will induce lysis in B. subtilis. Mutants have been isolated from original sensitive strains which are resistant to induced lysis.

Cell-free extracts of lysable B. subtilis have been observed, in the absence of any substrate, to liberate large amounts of NH_3 when incubated at 37°. It was determined that this NH_3 arose as a result

Part B included: Yes [x] No [ ]

of the intense autoproteolysis of the extracts. It was found that the cysteamine compounds sharply inhibited the production of NH_3. This inhibition obviously can occur either as a result of inhibition of the autoproteolytic process or as a result of inhibition of deamination of amino acids arising as a result of the proteolytic activity of the extracts. By testing the deaminase activity of the extracts versus 20 amino acids and by paper chromatography, it was found that the inhibition of NH_3 production by the cysteamine compounds was as a result of the inhibition of autoproteolysis.

It should be mentioned that the same specificity of compound applied in inhibition of proteolysis as in the lytic phenomenon.

Further investigation into this problem has been hampered by the extreme variability of B. subtilis strains in culture.

Significance to the program of the Institute: It is believed that studies with this group of compounds, about whose metabolism and physiology practically nothing is known, may lead to significant advances in our knowledge of cell physiology. That a substance such as cysteamine, for example, is of great importance to the living cell is obvious not only from the fact that it can prevent radiation injury but that it is also a portion of such biologically active molecules as cysteine, glutathione, thiamine, biotin, coenzyme A, and penicillin.

The cysteamine group of compounds comprises the most active substances discovered to date that protect mammals from radiation injury. A large number of additional compounds are to be tested to determine the extent of correlation between the bacteriolytic and radiation protective properties. If a good correlation exists, it should be possible to discover additional protective compounds by using the very rapid and inexpensive test for bacteriolysis as an initial screening method.

Proposed Course of the Program: The mechanism of lysis induced by cysteamine and related compounds, the mechanism of inhibition of autoproteolysis in B. subtilis by these compounds and the relationship, if any, between lysis and autoproteolysis will be investigated.

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Part B: Honors, Awards, and Publications

Weinberg, Eugene D., Saz, Arthur K., and Pilgren, Elisabeth Y.: Induction of bacteriolysis by cysteamine and its derivatives. J. Gen. Microbiol. 19: 419-433, 1958.

Serial No. NIAID 62
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Radioactively-Tagged Iron-Conalbumin as Antigen.

Principal Investigator: Dr. Arthur L. Schade

Other Investigator: Mr. Robert W. Reinhart

Cooperating Units: None

Man Years (calendar year 1958)
Total 6/12
Professional 3/12
Other 3/12

Project Description:

Objectives: To investigate the immunological activity of Fe^{59}-labelled conalbumin in animals and to correlate this activity with observable deposition of the antigen in the various organs of the host.

Methods Employed: Rabbits serve as the reactive hosts. Tagging of the conalbumin with iron accomplished by use of our published methods. Gamma ray scintillation counting employed in the location and quantitative estimation of the tagged antigen.

Major Findings: Fe^{59}-tagged conalbumin has been administered to actively- and passively-immunized rabbits and the fate of the antigen followed in the blood, liver, kidney, spleen, lung, and muscle over different periods of time.

Some of the conclusions that can at present be safely drawn, follow:

1. Response of both actively- and passively-immunized animals to the antigen was similar in all respects tested.

Part B included: Yes [ ] No [X]

2. The concentration of tagged antigen in the blood of immunized animals falls within one hour to 5 - 10% of its initial value. In 5 minutes, the concentration was reduced to approximately 20% and so a rapid removal of the antigen from the circulating blood is indicated.

3. Within six minutes at least 50% of the total administered antigen dose was located in the lung; after one hour only 25-35% remained. Conversely, only approximately 30% of the total dose was found in the liver after six minutes; after one hour this value had risen to 50%.

On an organ dry weight basis, the lung is the most active of the organs for removal of circulating antigen-precipitating antibody complex from the blood. Compared to the liver which is the only competitor for this distinction, ten times more antigen is immediately removed from the blood by the lung, gram for gram, than by the liver. Hence, it appears that the lung tissue operates as the initial accumulator of this antigen-precipitating antibody complex and in time leases the complex for accumulation elsewhere, primarily in the liver.

4. The distribution of the antigen antibody complex in the other body tissues show no unexpected features.

Significance to the Program of the Institute: The use of readily tagged antigens, whose integrity is not affected by the procedure of tagging, provides means for intimate study of antigen-antibody reactions, antigen localization in body tissues, and association of such localization with the organs involved in antibody production, asthmatic conditions, anaphylactic shock, allergic responses to infection, etc.

Proposed Course of Project: The data accumulated are being evaluated and readied for publication. We do not plan immediate work with Fe^{59}-conalbumin, unless evaluation of our present results so dictate. When undenatured pure siderophilin is available for use as an Fe^{59}-tagged antigen, we wish to make parallel studies with this iron-chelating protein.

Serial No. NIAID 63
1. Infectious Diseases
2. Medical and Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. Robert C. Woodworth
Mr. Robert W. Reinhart

Cooperating Units: National Cancer Institute
(Dr. James M. Stengle) NCI Serial No. 4-714(C)

Man Years (calendar year 1958)

Total	24/12
Professional	9/12
Other	15/12

Project Description:

Objectives: To investigate the contribution of blood serum siderophilin to the natural resistance of the host; to investigate the iron requirements of selected pathogens and the effect of the iron-chelating siderophilin and conalbumin on in vivo iron accumulation, growth, and metabolism of these pathogens; to investigate the factors leading to changes in concentration of both serum iron and siderophilin in circulating blood as a result of infection and other abnormal conditions, and the consequence of such changes on resistance to infection, iron absorption by the host and anemia.

Methods Employed: Bacterial cultural procedures in specially deironized media; spectrophotometric and radioisotopic methods; metabolic and enzymatic analytical procedures; and methods particularly suited to estimation of iron chelated either to siderophilin or conalbumin and to their relative unsaturations.

Major Findings: On the basis of previous findings showing that many bacteria will not grow in serum unless the contained siderophilin is saturated with iron, the conclusion was drawn that with

Part B included: Yes ☐ No ☒

respect to such bacteria the iron nutritional condition obtaining in the blood (extremely low concentration of free iron) provided an effective non-specific immunity barrier to infection by these bacteria irrespective of other possible specific or non-specific immunity factors. In addition, the rate of growth of some bacteria is decreased by the presence of unsaturated siderophilin. Similar results are obtained when the iron-binding conalbumin of egg white is present in the medium.

Employing Staphylococcus aureus, a pathogen whose growth in serum is not inhibited completely by unsaturated siderophilin, we have discovered that both siderophilin in serum and conalbumin in egg white or purified conalbumin in a growth medium are determining factors in the amount of iron incorporated into the cells. With a given concentration of either iron-chelating protein in the medium, the amount of iron taken up by the cells is a function not of the absolute iron concentration but of the square root of the ratio of the concentrations of the iron saturated to that of the iron-free iron-binding proteins. For example, in serum samples in which the siderophilin is 50% and 95% iron-saturated, the Staph aureus cells incorporate an amount of iron approximately 4.36 times as much at 95% saturation than at 50% saturation rather than only 1.9 times as much. This fact is of great significance for evaluating the contribution of siderophilin and its percentage of iron-saturation to its growth inhibiting properties for potential pathogens as well as to its role in the iron absorption and the overall iron metabolism of the host. Since the rate of growth of cells of Staph aureus as well as their metabolic activity are also some function of the percentage of iron-saturation of siderophilin (not yet fully investigated) and since the amount of iron absorbed from the intestinal tract in addition to that which can be made readily available to the bone marrow and other iron-requiring organs or tissues are functions of this same iron-chelate behavior, it is imperative that the physiological and physico-chemical properties of this serum component be fully investigated.

Practical use of these findings, employing Staph aureus as a test organism, has been made to determine the integrity of siderophilin isolated in a pure state from serum. For many clinical purposes, the investigators wish to have native, iron-active siderophilin for animal and human injections. Up to now, dissatisfaction has been experienced with the commercially available siderophilin employed in such studies. Although the products show iron-binding and appear homogenous and electrophoretically satisfactory, they do not function with respect to iron exchange in the same manner as do fresh normal sera. Where the Staph aureus iron incorporation test has been applied by us to samples of such purified siderophilins, we have found that they do not function as do fresh sera and thus have established reason for the dissatisfaction with the purified samples used in clinical tests.

From investigations on egg white and samples of purified conalbumin, we found that these samples likewise failed to behave normally in the Staph aureus test. By our development of a new method of conalbumin purification we succeeded in obtaining this iron-chelating protein in such a state that it functions normally with respect to iron exchange and in all other appropriate tests. This result encourages us to attempt the isolation of siderophilin from serum in an equally undamaged or undenatured state.

In a study with rabbits to elucidate, if possible, the phenomenon of serum iron and bilirubin rise in serum following intravenous administration of nicotinic acid as occurs in humans, we treated rabbits with acetyl-phenylhydrazine to establish an initial low serum iron level. Among other results obtained we wish to report that, concomitant with a fall in serum iron to 33% of its initial value, the total iron-binding capacity rose to 150% of its normal value. This increase of 50% of circulating siderophilin was accompanied by a fall in albumin concentration from 53% to 44%. We have no information to establish that the fall in albumin and rise of siderophilin levels are causally related but it appears likely that their behavior is part of a complex of reactions associated with greatly accelerated hematopoiesis.

Significance to the Program of the Institute: Believing that the siderophilin concentration, the serum iron level, and the metabolism of the iron itself in vivo all contribute, to the defense mechanisms of the host against infection, we wish to elucidate in every way possible their characteristics and changes under controlled conditions.

Proposed Course of Project: We propose to extend our investigations into the iron requirements of Staphylococcus aureus for its growth and metabolism and the control of the availability of such iron in serum mediated by siderophilin. We wish to analyze the components of these cells for their affinity for iron and the function of the iron in such components. For organisms, normally possessing hemin- containing respiratory enzymes, we wish to know whether there is a shift in the hemin-enzyme concentration or a qualitative change in the operative type of respiratory system. We wish to pursue the search for iron-active purified siderophilin by application of our Staph aureus iron accumulation procedure. With conalbumin and with siderophilin (when available) we wish to investigate the effect of increasing the percentage of iron unsaturation in the circulating blood of appropriate animals in the course of bacterial infection. We propose to make in vitro studies in serum of the iron utilization capabilities of splenic, liver, and bone marrow tissue from normal and infected rabbits to elucidate the serum iron behavior and anemia peculiar to infection.

Serial No. NIAID 64
1. Infectious Diseases
2. Medical and Physiological Bacteriology

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chemical aspects of the specific binding of iron to serum siderophilin and egg white conalbumin.

Principal Investigator: Dr. Robert C. Woodworth

Other Investigators: Dr. Arthur L. Schade
Mr. John R. Miller

Cooperating Units: None

Man Years (calendar year 1958)

Total:	27/12
Professional:	12/12
Other:	15/12

Project Description:

Objectives: Based to some degree upon the extensive studies of the hen's egg white iron-binding-protein, conalbumin, the goals of this project are to investigate the chemistry of the reactive groups in the siderophilin molecule responsible for the binding of iron and to determine the physical and chemical means by which the iron-siderophilin complex can be dissociated under physiological conditions obtaining in pathologic and normal hosts.

Methods Employed: Methods include the employment of radioactive tracer techniques, polarography, spectral analyses, ion-exchange chromatography, electrophoresis, polarimetry, and immunologic analyses for purity and for content of the iron-binding proteins.

Major Findings: A rapid, high-yield preparation of hen's eggs conalbumin has been worked out in this laboratory and has been accepted for publication in "Archives of Biochemistry and Biophysics." The method makes possible the preparation within a work week and in 82% of

Part B included: Yes [x] No [ ]

theoretical yield, conalbumin containing less than 0.001% lysozyme, 0.03% or less albumin, and a bound iron content 0.5% or less of total iron binding capacity. The conalbumin is at no point subjected to potentially denaturing subjects, such as alcohol. Studies of iron uptake by Staph. aureus from conalbumin thus prepared indicate that, as far as iron-binding is concerned, the protein is as "native" as it is in whole egg white.

The fact that all the bound iron of siderophilin or conalbumin is precipitated by the appropriate rabbit antiserum (as determined by tagging the iron with Fe^{59}), makes possible more precise quantitative calibration of rabbit anti-serum antibody content than is possible by the usual analysis for protein nitrogen. Furthermore, siderophilin and conalbumin are antigenically different, so that one may be precipitated quantitatively with no coprecipitation of the other. These findings have made it possible to study the exchange of iron between these iron-binding proteins. Preliminary results have shown that although under physiologic conditions of ionic strength and pH no free ionic iron is demonstrable, iron does indeed exchange from one protein to the other at a finite, measurable rate.

Comparisons of siderophilin samples purified by two different methods have shown the proteins to be significantly different from siderophilin in serum in regard to their iron-binding properties. These findings probably invalidate these preparative techniques for our purposes.

Significance to the Program of the Institute: The shifts in bound iron levels occurring as a result of allergic reactions and infectious diseases are recognized. Further, the availability of serum iron to the infective bacterial pathogen is a function of the siderophilin molecule's affinity for iron and of its degree of iron-saturation. Knowledge of the chemical bases of this affinity should elucidate the manner in which siderophilin governs the supply of iron to the host as well as to the pathogen.

The availability of pure, undenatured, actively iron-binding siderophilin would be of considerable value for in vivo studies of the effect of increased iron-free siderophilin (and therefore decreased iron saturation) on allergic states, infections, and iron absorption in normal and anemic conditions.

Proposed Course of Project: We propose to pursue further the possibility of preparing pure siderophilin from blood plasma or serum without altering the protein's iron-binding properties.

Detailed study of the rates of iron uptake and release by the iron-binding proteins should yield valuable information concerning the chemical equilibria between these proteins and iron and lead to further understanding of the relative availability of iron to host and pathogen.

Further understanding of the intimate chemistry of the iron-protein complexes will come from study of (1) the stoichiometry of the iron-protein-carbonate complex, (2) the location and nature of the proteins' iron-binding sites and (3) the stereochemical requirements of the protein molecule for the binding and dissociation of iron.

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Part B: Honors, Awards, and Publications

Woodworth, Robert C. and Schade, Arthur L.: Conalbumin: A Rapid High Yield Preparation from Egg-White.

Accepted for Publication, Archives of Biochemistry and Biophysics.

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1. Infectious Diseases
2. Medical and Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chemotherapy of Potential Tuberculostatic, Fungistatic, Parasiticides and Viricidal Agents. Detoxification Studies.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: Miss Gladys Kauffmann
Mr. Herald J. Stone

Cooperating Units: None

Man Years (calendar year 1958)
Total: 19/12
Professional: 12/12
Other: 7/12

Project Description:

Objectives: Further studies on toxicity and detoxification of streptomycin (SM), isoniazid (INH) and mixtures of streptomycin-isoniazid (SM-INH) in two strains of mice. To find means of reducing further the toxicity of these drugs separately and in mixtures without interference with bacteriostatic activity.

Methods Employed: The effect of various vitamins, vitamin analogs, amino acids and calcium salts on the toxicity of INH and or SM was measued by a) the determination of survival times of two strains of mice following simultaneous subcutaneous administration of sublethal and lethal amounts of the drugs with each adjuvant, and b) blood plasma levels of INH as measured by the "pyridyl" method. The in vitro bacteriostatic action of the drug-adjuvant combination was also determined.

Major Findings: 1. With vitamins survival was 7% with white mice and 12% with DBA mice. Simultaneous administration of the dose of SM with an equimolar amount of each of these adjuvants, calcium pantothenate, nicotinic acid or ascorbic acid increased survival to 90 per cent. On a molar basis, it required 8 to 10 times as much of the adjuvants p-amino-benzoic, inositol or isonicotinic acid for the

Part B included: Yes [X] No [ ]

detoxification of a similar dose of SM. Up to 20 daily doses of the SM adjuvant mixture with calcium pantothenate or nicotinic acid were tolerated by 80 to 90% of the mice tested. The antibiotic-vitamin combinations were as inhibitory as the SM alone or in vitro bacteriostatic tests on an avirulent strain of tubercle bacillus.

2. With the vitamin analogs: Test of appropriate amounts of two sulfonic acid analogs of vitamins, pyridine-3-sulfonic acid and sulfanilic acid as well as possible detoxifying agents for streptomycin were carried out in two strains of mice. With a 10 mg dose of SM, which is 93% lethal in white mice and 88% in DBA mice, pyridine-3-sulfonic acid as adjuvant in a 10 mg dose permitted 85% survival of white mice and in a 2.5 mg dose permitted 82% survival of DBA mice.

When injected simultaneously with 25 mg of the adjuvant the SM dose may be increased to 15 mg with 65% survival of white and 100% of DBA animals. With sulfanilic acid and 6-thymol-sulfonic acid the results were very similar, with the outstanding exception that 20 mg SM could be tolerated with 50 mg of sulfanilic acid by 60% of the white and 80% of the DBA mice. In chronic toxicity tests, sulfanilic acid appeared to be the one of choice under the conditions of the test.

3. With L-cysteine HCl: The use of appropriate amounts of this amino acid as adjuvant permitted 19 of 20 white mice to tolerate a lethal mixture of 10 mg SM and 2 mg INH when administered subcutaneously. On single administration this amino acid in a dose of 58 mg was effective in permitting survival of mice given 40 mg SM, 8 times the maximum tolerated dose when given alone.

4. With calcium chloride or calcium lactate: Successful detoxification in acute toxicity tests in mice have been repeated with calcium pantothenate-streptomycin mixtures. Discussion has arisen as to the role of the Ca ion in the pantothenate salt. In this connection, calcium chloride and calcium lactate have been tested. Each salt proved effective in permitting mouse survival when administered simultaneous with a toxic dose of SM (10 mg). The effective doses of the salts were 1.9 mg calcium when given as the chloride (5 mg), or 0.92 mg when administered as the lactate (5 mg).

5. Vitamins with SM-INH mixtures. 14 vitamins have been tested as adjuvants with various mixtures of the two therapeutic agents in two strains of mice. 5 mg of SM or 2 mg of INH are nontoxic per se by subcutaneous injection. However, a mixture of these concentrations permitted only one-third of the animals of either strain to survive. With vitamins as adjuvants in such a mixture, each of 11 were effective in white mice and each of four in DBA mice, permitting 90 to 100% survival. Calcium pantothenate and nicotinic acid were 100% and 90% effective in detoxifying double the amounts of the toxin mixture of therapeutic agents (10 mg SM + 4 mg INH) in white mice and 42% effective in DBA mice.

Significance to the Program of the Institute: The widespread use of the chemotherapeutic agents streptomycin and isoniazid in the treatment of human tuberculosis is limited by their toxicity. Hence means of increasing the tolerance should permit more effective therapy.

Proposed Course of the Project: This study will be extended to include several new compounds that show promise as detoxifying agents.

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Part B: Honors, Awards, and Publications

Prescott, B., Kauffmann, G., and James, W. D.: Means of increasing the tolerated dose of streptomycin in mice. Antib. and Chemo. 8: 27-32, 1958.

Prescott, B., Kauffmann, G., and James, W. D.: Further studies on the effect of glycerine on toxicity of isoniazid-streptomycin mixtures in mice. Antib. and Chemo. 8: 255-262, 1958.

Prescott, B., Kauffmann, G., and James, W. D.: Effect of glycerine on toxicity of isoniazid-streptomycin mixtures in mice. Antib. and Chemo. 8: 81-83, 1958.

Prescott, B., Kauffmann, G., and James, W. D.: Means of increasing the tolerated dose of isoniazid in mice. IV. Certain keto acids. Antib. and Chemo., 8: 349-353, 1958.

Prescott, B., Kauffmann, G., James, W. D., and Stone, Herald J.: Increase of the tolerated dose of isoniazid in mice by use of certain organic solvents. Antib. and Chemo., 8: 470-475, 1958.

Serial No. NIAID 66
1. Infectious Diseases
2. Medical and Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Detoxification of parasiticidal drugs

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: Miss Gladys Kauffmann
Mr. Herald J. Stone

Cooperating Units: Section on Chemotherapy, LED-NIAID
(Dr. Geo. Luttermoser) Serial No. 122-D
(Dr. Jos. Greenberg)
(Dr. C. Robt. Coatney)

Man Years (calendar year 1958)
Total: 9/12
Professional: 6/12
Other: 3/12

Project Description:

Objectives: To find means of reducing the toxicity of the widely used parasiticidal drugs.

Methods Employed: Various known detoxifying agents were studied for their ability to detoxify toxic doses of parasiticides. The agents were given simultaneously with parasiticides to infected animals.

Major Findings: With Miracil D - various amino acids, vitamins, sulfonic acids, alpha keto acids and organic solvents have been tested orally simultaneously with this drug. 20 gm white mice will tolerate only 5 mg (250 mg/kg) of Miracil D orally for 5 days. With a mixture of 30 mg of Miracil D and 50 mg 6-thymolsulfonic acid, mice survived 15 daily doses. 70 mg of Miracil D with 50 mg of 6-thymolsulfonic acid could be tolerated by 100% of the mice for 3 daily ad-

Part B included: Yes [ ] No [x]

ministrations. With Hoechst 688, 40 mg of the drug administered orally in one dose to a 20 gram mouse was lethal. When mixed with 50 mg L-cysteine-HCl, 100% of white mice survived. On repeated daily administration, this dosage combination was tolerated for 15 doses by 70% of the animals.

With Heterazan: With glycine and glucuronic acid at levels of 50 mg each per 20 gram mouse, 70% of the mice tolerated administration of 40 mg of Heterazan (lethal per se) for 12 daily doses. The same results were obtained with 6-thymolsulfonic acid as adjuvant. With 35% glycerine as adjuvant, 80% of the animals survived 15 daily administrations of the lethal 40 mg dose.

With chloroquine, several aromatic sulfonic acids were effective in increasing the tolerated dose of this drug, permitting mice to survive 3 to 6 times the lethal dose. DBA mice survived a 40 mg oral dose when combined with 50 mg of sulfanilic acid or a 50 mg dose with 75 mg of sulfanilic acid. 83 per cent of the mice survived a mixture of 60 mg of chloroquine and 100 mg of sulfanilic acid. Of 5 additional sulfonic acids similarly tested, four showed excellent detoxifying action, i.e., 6-thymolsulfonic, orthanilic, metanilic, pyridine-3-sulfonic and dl-10-camphosulfonic acids. The first gave optimal results when 50 mg was mixed with 50 mg of chloroquine.

Daraprim: With a lethal 2.5 mg oral dose, solution in 50 per cent aqueous propylene glycol permitted 70% survival of mice on repeated administration for 15 days with no toxic manifestations.

Primaquine: A 5 mg/20 gram mouse lethal dose of primaquine is tolerated by 80% or more of white mice when administered orally simultaneously with 25 mg of 6-thymolsulfonic acid or by 70% of the animals with 50 mg sodium glucuronate and 50 mg glycine.

Pamaquine: Of 36 adjuvants tested, two showed detoxifying action, namely folic acid and 6-thymolsulfonic acid. On single oral administration of a 2.5 mg lethal dose of pamaquine per 20 gram mouse, 84% of the mice survived when 25 mg of 6 thymolsulfonic was used as adjuvant and 50% survived twice the lethal dose with 50 mg 6-thymolsulfonic acid. With the 2.5 mg lethal dose and 5 mg of folic acid as adjuvant 35% of twenty mice survived 20 daily administrations.

Pentaquine: The aromatic sulfonic acid, (6-thymolsulfonic) was effective in increasing the tolerated dose of the drug, permitting 100% of two strains of mice to survive six times the lethal dose.

Oropentaquine: The results with this drug were very similar to pentaquine.

Significance to the Program of the Institute: Greater therapeutic effectiveness against human infections conceivably could be achieved if dosages of the toxic chemotherapeutic agents were increased, without loss in chemotherapeutic activity. Massive doses of chemotherapeutic agents could result in decreased rates of emergence of resistant strains thus leading to more effective therapy.

Proposed Course of the Project: In addition to the indicated procedures with the above studies, this study will be extended to determine the mode of action of reversal of the detoxifying agents that show promise. Since Miracil D though toxic was reported to have anti-tumor activity, the non-toxic complex of Miracil D and 6-thymolsulfonic acid will be tested for its anti-tumor activity.

Serial No. NIAID 67
1. Infectious Diseases
2. Medical and Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Potential Fungistatic Agents

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: Mr. Herald J. Stone

Cooperating Unit: Section on Mycology, LID-NIAID (Dr. Chester W. Emmons, NIAID 73)

Man Years(calendar year 1958)
Total: 6/12
Professional: 3/12
Other: 3/12

Project Description:

Objectives: Synthesis and Tests of Potential Chemotherapeutic Agents against Pathogenic Fungi. To isolate effective, non-toxic compounds for use in therapy of pathogenic fungi.

Methods Employed: A series of 50 thymol derivatives were synthesized by condensing various aromatic aldehydes, isocyanates and isothiocyanates with p-amino thymol in absolute ethyl alcohol. The final recrystallized products were analyzed for carbon, hydrogen, nitrogen and tested for melting points. Toxicity studies were performed in white mice.

Major Findings: Of the 50 crystalline products synthesized, none was as toxic as mycostatin or Amphotericin B, the currently used fungistatic agents.

Significance to the Program of the Institute: Because of higher tolerance, these compounds may be useful as potential fungistatic agents.

Proposed Course of the Program: Tests for usefulness of these compounds are being run to determine their effectiveness in experimental fungal infections in laboratory animals.

Part B included: Yes ☐ No ☒

Serial No. NIAID 68
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Colorimetric Method for Determination of Kanamycin in Blood Plasma.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: Mr. Herald J. Stone

Cooperating Units: None

Man Years (calendar year 1958)
Total: 6/12
Professional: 3/12
Other: 3/12

Project Description:

Objectives: Development of a simple, direct quantitative chemical method for the assay of the new antibiotic and tuberculostatic agent Kanamycin in blood, tissues, urine and biological fluids of animals. The evaluation of the effectiveness of this drug in staphylococcal infections, experimental tuberculosis in animals and in human tuberculosis requires basic knowledge of blood levels, tissue levels and excretion following the various routes of administration.

Methods Employed: A review of existing methods revealed time-consuming microbiological procedures. A new chemical method and procedure is being developed utilizing the observation that the compound 2,3-dichloro-1,4-naphthoquinine produces a colored compound with the hydrolysis products of Kanamycin.

Major Findings: A chemical method has been developed which detected levels of Kanamycin of 50 gamma/ml. in blood plasma.

Significance to the Program of the Institute: This method is applicable to blood from experimental animals, permitting basic studies in chemotherapy.

Proposed Course of the Project: Study is being continued to provide a more sensitive method.

Part B included: Yes [7] No [X]

1. [illegible]
2. [illegible]
   bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1956

Part A

Project Title: Studies of Upper Respiratory Tract Bacterial Flora of Young Children

Principal Investigator: Dr. Roger M. Cole

Other Investigators: Edythe J. Rose; Dorothy E. Moore; Norman S. Ikari

Cooperating Units: Epidemiology Section, LID, NIAID, NIH

Man Years:
Total: 4-1/3
Professional: 1-1/3
Other: 3

Project Description:

Objectives:

To observe the occurrence, both endemic and epidemic, in sickness and health, of several pathogenic bacteria in the upper respiratory tracts of young children.

Methods Employed:

Direct throat swabs are taken routinely once weekly from a nursery population up to three years of age, as well as at times of admission, illness, and discharge. Platings are made on appropriate media, incubated, and relative numbers of the various bacteria recorded. Colonies of beta-hemolytic streptococci, Hemophilus spp., pneumococci, and beta-hemolytic staphylococci are identified and picked for purification

Part B included Yes [X] No [ ]

Further identification is accomplished biochemically and by specific antisera for streptococci, pneumococci, and Hemophilus influenzae. Staphylococci are identified by phage typing. Bacterial strains isolated are preserved by lyophilization and added to the collection for future reference. A record of each preserved strain is kept on keysorter punch made cards, and a similar annotated individual card is kept for each culture result on each child. Results are being tabulated and analyzed by various attributes.

Major Findings:

During the calendar year, throat cultures, both routine and non-routine, were taken during the first 6 months. The 26-week period was terminated on June 28, ending 1½ years of bacteriological experience in the nursery population of Junior Village. Cultures in the 26-week period totalled 2,123: 1,617, taken on routine days, and 506 taken because of admission, illness, or discharge.

Of the total, 272 (13.8%) were positive for streptococci of Group A (including No Type, Type 12, and Type 5). These isolations were recorded in relatively sharp outbreaks by each serologic type. In contradistinction to the lack of discrete and limited persistence of types of other bacteria found. Few isolations of Groups C and G (18 and 6, respectively) were made.

Hemophilus influenzae, Type B, was isolated from 416 cultures (19.6%); other iridescent H. influenzae were found only 19 times.

Pneumococci, comprising 10 different serologic types, were isolated from 215 cultures (10.1%). Type 19, which had been initially most prevalent, was later replaced by Type 23.

The phage typing of staphylococci isolated during the entire 1½ year study was completed. In 1958, 562 isolations of staphylococci were made: 217 were not further identified; and 345 (16.3%) were coagulase-positive, beta-hemolytic organisms that were subjected to phage typing. Of these, 18 were No Type and 7 were assorted phage types: the remaining 313 isolations were phage type 81 or combinations of types including 81. Most abundant was type 42B, 81, the so-called "epidemic type", which was isolated 237 times and thus accounted for 68.7% of the typed staphylococcus isolations.

Routine bacteriological surveillance of this population has been completed and detailed analyses are in progress. These will include prevalence and incidence of the various bacteria; the effects thereon of penicillin prophylaxis; variations therein by week and their relation to the incidence of febrile illness onsets; and eventual correlation with the simultaneous occurrence of various viruses. The crude data suggest that many bacterial pathogens may be readily isolated from throats of young children for long periods of time without definitive overt relation to clinical disease, either individually or epidemiologically.

Significance to the Program of the Institute:

Little data is available concerning the sequence of infection with various micro-organisms in childhood; the duration of such infections; their relation to disease; and the effects of simultaneous infection with multiple agents. It is expected that this study will supply information pertinent to the above and will indicate in proper perspective the microbiologic experience of young children.

Proposed Course of Project:

Routine bacteriological field work terminated; statistical analysis and indicated laboratory studies of bacteria isolated to continue.

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3. Bethesda, Maryland

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Emmart, E.W., Cole, R.M., May, E.L., and Longley, J.B. Studies on Streptococcal Hyaluronidase and Anti-hyaluronidase. II. The Localization of Sites of Absorption of Streptococcal Hyaluronidase (Group C) With Fluorescent Antibody. The Journal of Histochemistry and Cytochemistry, 6:(3),161-173, May, 1958.

Honors and Awards relating to this project:

None

Serial No NIAID 70
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Investigations of the Type-specific Immunity to Group A Streptococci

Principal Investigator: Dr. Fred S. Kantor

Other Investigator: Dr. Roger M. Cole

Cooperating Units: None

Man Years:
Total: 1-2/3
Professional: 1-1/3
Other: 1/3

Project Description:

Objectives:

To improve methods of production and detection of Type-specific antibody to Group A Streptococci; and to determine the relationship between enzyme-extracted "M" protein and that material prepared by other methods with especial reference to their respective antigenicities.

Methods Employed:

A streptococcal cell-wall enzyme (lysin) is prepared from fresh phage lysates of Group C streptococci. This lysin is used to hydrolyze Group A streptococci cell walls and the

Part B Included Yes [ ] No [X]

resulting digest is fractionated by means of ammonium sulfate precipitation and ion exchange column chromatography. The "M" protein prepared by these methods is compared, with respect to antigenicity in rabbits, with "M" protein extracted by conventional means. For this purpose standard mouse-protection tests, bactericidal properties, and precipitin analyses are used.

Major Findings:

Lysin prepared from phage lysate will separate "M" protein from Group A cell walls. This material has been found to be highly antigenic in rabbits, although the nature of the antibodies produced is not yet clear.

Significance to the Program of the Institute:

Antibody to "M" protein appears to be necessary for type-specific immunity in humans. To date the antigenicity of previous preparations of this material has been poor. Solution of this problem will clarify the role of "M" antibody in type-specific immunity and make streptococcal immunization more feasible.

Proposed Course of Project:

Continuation in direction indicated.

It is desirable to investigate some of the environmental factors which may affect transduction, i.e., (1) media changes (2) presence of extracellular DNA slime layer in possible recipients (3) elaboration of extracellular DNase by possible recipients (4) lysogenic phage in donor strains.

Attempts will be made to transduce some of the "virulence" properties such as toxin production and coagulase of pathogenic strains to innocuous staphylococci.

Major Findings:

None to date. Work on this study was initiated in late summer, 1958, and has involved production of stable mutants, finding suitable marker media and producing and quantitating phage lysates.

Significance to the Program of the Institute:

Genetic transfer by DNA and phage transduction has been profitably investigated in several bacterial genera but not extensively in staphylococci. Published reports in work of this type with staph probably number less than half a dozen to date. Since staphylococci have assumed increasing importance in respect to its troublesome transmissible characteristics, its ubiquitousness and antibiotic resistance, investigations leading toward elucidation of any segment of its basic genetic nature would be helpful.

Proposed Course of Project:

Continuation in direction indicated.

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Individual Project Report
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Serial No. NIAID-72
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

Project Title: Ecology of Pathogenic Fungi.

Principal Investigator: Dr. Chester W. Emmons

Other Investigator: Mr. Willard Piggott

Cooperating Units: None

Man Years (calendar year 1958)
Total: 2
Professional: 7/12
Other: 1 5/12

Project Description:

Objectives:

To discover the natural habitats of pathogenic fungi and the factors which determine their distribution in soil; to resolve the confusion existing in published papers regarding the etiology of certain "epidemics" of mycoses; to seek new and significant ecological associations of pathogens.

Methods Employed:

Search of environments of patients with mycoses; collection and processing of soil samples by mouse inoculation; periodic sampling of soils from premises known to be contaminated with pathogenic fungi; study of microfloras of these soils.

Major Findings:

A new and apparently highly significant association between Histoplasma and the house bat at one home with one fatal and several

Part B included.

clinical cases of histoplasmosis has been confirmed by observation of similar associations at two other sites. We continue to isolate Cryptococcus with regularity from buildings (both rural and urban) which shelter pigeons.

Significance to the Program of the Institute:

These studies have elucidated some of the epidemiological puzzles associated with mycoses. The almost constant association of virulent strains of Cryptococcus with pigeons has important public health implications.

Proposed Course of Project:

These studies hold great further promise and will be continued.

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Emmons, C. W. Association of bats with histoplasmosis. Public Health Repts. (U.S.), 73:590-595, July 1958.

Emmons, C. W. The organism, Histoplasma capsulatum. Proc. of Histoplasmosis Seminar, The Jewish Hospital Association, Cincinnati, Ohio, pp 2-7, Feb. 11, 1958.

Emmons, C. W. Fungus nuclei in the diagnosis of mycoses. Mycologia. (In press).

Emmons, C. W. Environmental sources of infections in the mycoses. Proc. VI Int. Cong. on Trop. Med. and Malaria. (In press).

Emmons, C. W. Saprophytic reservoirs of Histoplasma. Histoplasmosis. Chapter in book (in press), edited by H. C. Sweaney).

Emmons, C. W. Medical Mycology. Otolaryng., Vol. IV. W. F. Prior Company, Inc., Hagerstown, Maryland. (In press).

Louria, D. B., Mitchell, W. and Emmons, C. W. The influence of the time of animal sacrifice and the volume used for direct culture on the isolation of pathogenic fungi. J. Lab. & Clin. Med. (In press).

Emmons, C. W. Revision of mycology sections of "Control of Communicable Diseases in Man", A.P.H.A. Handbook. (In press).

Honors and Awards relating to this project:

President-Elect of the Mycological Society of America.
Appointed Rapporteur for the arrangement of the Medical Mycology Program of the International Botanical Society, Montreal, Canada, 1959.
Member, Bacteriology and Mycology Study Section, NIAID.
Corresponding Member, Soc. Belge Med. Trop.
Member of Advisory Council, American Type Culture Collection.

Part B (continued).

Editorial Board: Journal of Bacteriology
Mycologia

Professorial Lecturer, G. W. Hospital School of Medicine.

Vice-President, International Society for Human and Animal Mycology.

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Serial No. NIAID-73
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

Project Title: Chemotherapy of experimental mycoses.

Principal Investigator: Dr. Chester W. Emmons

Other Investigator: None

Cooperating Units: Cooperation with LID,NIAID (Dr. Prescott, Serial #67), in preparation of chemicals, and with (Dr. Treger, LCI,NIAID, Serial #23(c)), in clinical trials.

Man Years (calendar year 1958):

Total:	2
Professional:	5/12
Other:	1 7/12

Project Description:

Objectives:

To test promising drugs and antibiotics for toxicity and for *in vivo* activity in control of experimental mycoses.

Methods Employed:

Histoplasma, Coccidioides, Blastomyces and Cryptococcus are used as test organisms. Standardized infections are produced in mice by intravenous injection of measured inocula; treatment by varied routes is begun after 1 or more days. Effectiveness of drug is measured by extension of survival time of treated mice compared with untreated, and by culture of appropriate organs.

Part B included.

Major Findings:

One new antibiotic has been found effective and low in toxicity in mice. Other antibiotics and chemicals under test show promise sufficient to warrant further tests.

Significance to the Program of the Institute:

An antimycotic drug or antibiotic is needed for clinical use.

Proposed Course of Project:

No drugs or antibiotics presently available are entirely satisfactory for clinical use and promising new drugs or antibiotics therefore will be tested in the future as they become available.

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Serial No. NIAID-71
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A:

A. Project Title: Biochemistry and Physiology of Pathogenic Fungi. (In vitro studies of the action of Amphotericin B on pathogenic fungi).

Principal Investigator: Dr. George W. Jones

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1 4/12
Professional: 8/12
Other: 8/12

Project Description:

Objectives:

To investigate the quantitative relationships of this antifungal drug with respect to fungicidal and fungistatic action; to study certain physical and chemical factors influencing this action; to study the effects of this drug on certain metabolic activities of fungi; to study the emergence of strains resistant to this drug; and ultimately to understand the mechanism of action of this member of a group of related antifungal antibiotics.

Methods Employed:

Candida albicans is used as the test organism. The relationship of drug concentration to death rates, growth inhibition, respiration, and fermentation is studied by standard methods. Antibiotic sensitivities are determined in liquid culture photometrically. The effects

Part B not included.

of metabolites and related substance on drug sensitivity are determined.

Major Findings:

Cysteine and several sterols possess the ability to reduce the activity of Amphotericin B. The tolerance for Amphotericin B of all twelve strains of Candida albicans studied has been increased by prolonged growth in the presence of the drug. While most changes were small, three strains increased tenfold or greater, and one strain 29-fold. Several of the strains were found to be somewhat altered also in morphology, growth rate, and virulence for mice.

Significance to the Program of the Institute:

Amphotericin B is an antifungal antibiotic receiving considerable attention from clinicians for the treatment of mycoses. Knowledge of its properties, mode of action, and the emergence of strains resistant to it are of importance in the treatment of this increasingly significant group of diseases.

Proposed Course:

It is planned to continue these studies. The study of the quantitative relationships of the antagonism of Amphotericin B by cysteine and the sterols will be elaborated. The resistant variants of C. albicans will be used to study further the mechanism of action of this drug.

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Calendar Year 1958

Serial No. NIAID 75
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: Studies on the physiology of Coccidioides immitis.

Principal Investigator: Dr. George W. Lones

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	8/12
Professional:	4/12
Other:	4/12

Project Description:

Objectives:

The biochemistry and metabolism of this dimorphic pathogen have been little studied. It is the purpose of this project to obtain information on the metabolic characteristics of this organism, particularly in its parasitic form.

Methods Employed:

Shake culture techniques are employed to produce the parasitic phase of C. immitis in sizeable quantities for examination by standard chemical and biochemical techniques.

Major Findings:

The culture method of Converse has been adapted for the growth of a strain of C. immitis in large quantities and essentially free of the mycelial phase. This culture has been carried through 40 successive transfers with no apparent deterioration. Cells produced in this manner have been found suitable for the study of factors influencing

Part B not included.

respiration. Although this fungus does not grow anaerobically, it has been found capable of carrying out an alcoholic fermentation of glucose.

Significance to the Program of the Institute:

Progressive coccidioidomycosis is a disease with a high mortality. A better knowledge of the causative agent may favorably influence our diagnosis, prevention and treatment of the infection.

Proposed Course:

Successful cultivation of the parasitic form of C. immitis makes available adequate quantities for metabolic and chemical studies. Extracellular, capsule-like structures are being examined. Factors influencing aerobic and anaerobic metabolism will be investigated, and the parasitic and mycelial forms will be characterized metabolically.

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Serial No. NIAID-76
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Immunity Studies with Pathogenic Fungi.

Principal Investigator: Dr. H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	10/12
Professional:	5/12
Other:	5/12

Project Description:

Objectives:

To elucidate fundamental principles involved in development of acquired resistance to experimental sporotrichosis in laboratory animals, using this disease as an example of one type of mycoses.

Methods Employed:

Immunization procedures include the use of several vaccines prepared in various ways and injected according to a predetermined schedule. Animals are subsequently challenged with logarithmic dilutions of a known number of virulent yeast cells.

Major Findings:

Some protection against death in the "immunized" animals has been observed, however, "protected animals" are infected.

Part B not included.

Significance to the Program of the Institute:

These studies are contributing basic information about the immunology of mycoses.

Proposed Course:

The studies with sporotrichosis will be terminated after a few more experiments, and similar investigations will be undertaken using other mycoses as models.

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Serial No. NIAID-77
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

C. Project Title: Antigenic Studies on Yeasts

Principal Investigator: Dr. H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 6/12
Professional: 3/12
Other: 3/12

Project Description:

Objectives:

To try to learn more about the antigenic relationships existing among some of the provisional pathogens.

Methods Employed:

Rabbits are immunized with yeast cell suspensions, the immune serum collected, and adsorption procedures are followed. Thus far, tube agglutinations have been utilized in the antigenic analysis.

Major Findings:

The studies are still in the initial stages.

Significance to the Program of the Institute:

These studies should lead to fundamental information about specific antigens of fungi with the practical application of an improved and rapid method of identifying yeast isolates.

Part B not included.

Proposed Course:

The studies will be continued.

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Serial No. NIAID-78
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A

B. Project Title: Virulence and pathogenic studies with yeasts.

Principal Investigator: Dr. H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	8/12
Professional:	4/12
Other:	4/12

Project Description:

Objectives:

To gain more fundamental knowledge with regard to the disease producing characteristics of Candida albicans and Cryptococcus neoformans.

Methods Employed:

Comparative virulence studies of strains of C. albicans representing extreme ranges of variation in mice and rabbits have been made by the intravenous inoculation of known number of cells.

Experiments with C. neoformans have been designed to follow the course of progressive disease by determining by plating techniques the number of yeast cells in various organs from soon after inoculation until death of the experimental animals.

Part B not included.

Major Findings:

Studies with C. albicans have revealed great difference in virulence and have indicated that the mouse is at least as susceptible to candidiasis as the rabbit.

Investigation with cryptococcosis is still in the initial stages.

Significance to the Program of the Institute:

These studies are contributing basic information about the variations in strain virulence, the host relationship to mycotic diseases, and the interplay of these factors.

Proposed Course:

The studies will be continued.

Serial No. NIAID 19
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Epidemiological Studies of Newly Developed Vaccines

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Albert Z. Kapikian, Dr. Thomas F. Reichelderfer, Dr. John E. Craighead

Cooperating Units: Assistance from Virus and Rickettsial Section, LID, District of Columbia Welfare Department, and United States Navy Medical Research Unit No. 4

Man Years (calendar year 1958):
Total: 5 Man Years
Professional: 2 Man Years
Other: 3 Man Years

Project Description:

A. Basic Objectives

(1) The overall objective is to develop new vaccines which will have practical value for preventing common acute infectious diseases. This broad objective is in part covered by other projects (#80 and #81) viz., those concerned with finding new etiologic agents of disease and those concerned with evaluating the importance of such diseases. This project in particular is concerned with studying vaccine induced antibody responses, reactions, and protection against clinical diseases. Inactivated influenza, adenovirus, measles, Coxsackie B virus and Hemadsorption virus vaccines are being studied. Considerable effort is being expended to develop for future study multipolyvalent vaccines containing some 10-25 different virus types known or suspected to be etiologic agents of respiratory diseases.

(2) Patient material

(a) Mentally defective children 2 to 10 years of age at Laurel, Maryland, under domiciliary care of District of Columbia Welfare Department.

(b) Homeless, but otherwise normal children at Junior Village, Washington, D. C. (See Project #81)

(c) 2-10 year old children at Children's Convalescent Home, Washington, D. C.

(d) Naval recruits at Great Lakes Naval Training Center

ncluded

(e) Reformatory volunteers and Community populations as indicat

(3) Methods

New vaccine products are thoroughly tested in the laboratory for safety, purity, and potency, then are usually given to laboratory workers and a few children to confirm freedom from reactions and adequacy of antibody response. As indicated they are then used on a broader scale in suitable populations to determine protective value against naturally occurring disease.

(a) Asian influenza virus vaccine was used in controlled studies in volunteers and in children < 10 years of age at Laurel, Maryland, and at the Children's Convalescent Home and in < 3 year olds in the Junior Village Nursery group.

Vaccine responses were measured serologically. Influenza was recognized clinically by virus isolation and by serologic methods. One and two vaccine doses were used.

(b) Measles vaccine, three polyvalent adenovirus vaccines, and a polyvalent Coxsackie B virus vaccine were used in children at Laurel and antibody responses to one and two doses are being studied.

(c) Two polyvalent adenovirus vaccines, one polyvalent Coxsackie B vaccine and a measles vaccine are being used in controlled studies at Junior Village in an effort to ascertain their effectiveness in protection against infection and clinical disease. (See project # 81)

(d) A polyvalent adenovirus vaccine, a polyvalent influenza vaccine containing no Asian strain and a monovalent Asian strain influenza vaccine together with placebo controls were used in Naval recruits at the Great Lakes Naval Training Center to evaluate their effectiveness in protection against clinical disease.

Major Findings:

The inactivated Coxsackie B vaccine, measles vaccine, adenovirus vaccine and influenza vaccine produced no untoward reactions of consequence and all appear to induce substantial antibody response. Two doses of influenza vaccine induced a far better antibody response in children than a single dose and gave excellent protection against clinical influenza during a laboratory confirmed outbreak. In adult naval recruits, a single dose of monovalent Asian strain influenza vaccine gave excellent clinical protection against epidemic Asian influenza. The polyvalent influenza vaccine containing no Asian strain virus also gave clinical protection against epidemic Asian influenza but the small amount of protection indicated that effective influenza vaccine prophylaxis

requires vaccines containing virus strains antigenically similar to the epidemic strain. The adenovirus vaccine used routinely in Naval recruits continued to give substantial protection against ARD of recruits.

Significance to Program of the Institute:

It is significant that the Laboratory of Infectious Diseases first discovered the family of adenoviruses, first classified them, first established, beyond reasonable doubt, their etiologic role in disease, first prepared a vaccine and established its effectiveness and first demonstrated that a reproducible commercially prepared product would give substantial protection against acute respiratory disease and viral pneumonia so prevalent in recruits. It also first demonstrated effectiveness of Asian influenza vaccines. All of these findings have been confirmed by continued studies in this Laboratory and by extensive studies of other investigations in the field.

Proposed Course of Project:

It is planned to pursue this project in the manner described as altered by continually changing conditions as long as there is continued promise of developing effective prophylactic vaccines which are simple of administration and worthwhile for general use in preventing acute infectious illnesses -- particularly, those so common in childhood.

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Part B: Honors, Awards, and Publication

Publications:

Gundelfinger, B.F., Hantover, M.J., Bell, J.A., Loosli, C.G., Rowe, W.P.

"Evaluation of a Trivalent Adenovirus Vaccine for Prevention of Acute Respiratory Disease in Naval Recruits"

Amer. J. Hyg., Vol. 68, Page 156-168 (Sept. 1958, No. 2)

Gundelfinger, B.F., Stille, W.T., and Bell, J.A.

"Effectiveness of Influenza Vaccines During an Asian Influenza Epidemic"

New Eng. J. of Med., Vol. 259, No. 21, pp. 1005-1009

Honors: None

Awards: None

Serial No. MIAID 00
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

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Part A.

Project Title: Epidemiological Investigations of Acute Infectious Diseases

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Albert Z. Kapikian, Dr. Leon Rosen, Dr. Thomas E. Reichelderfer, Dr. John E. Craighead

Cooperating Units: Virus and Rickettsial Section LID, (Dr. Chanock Dr. Rowe, Dr. Huebner) and Lobund Institute of Notre Dame University (Dr. Thomas G. Ward)

Man Year (Calendar Year 1958):
Total: 7 Man Years
Professional: 3 Man Years
Other: 4 Man Years

Project Description:

A. Basic Objectives

(1) To study acute infectious illnesses as they occur in the hospitals, schools, institutions, and in the community-at-large;

(2) To recognize apparent clinical and epidemiologic entities, and to collect appropriate specimens for laboratory studies in an effort to discover new etiologic agents of disease;

(3) To study epidemiologic characteristics of such diseases;

(4) To evaluate their importance to the general public health and the need for research efforts toward their control.

Methods and patient material:

In general, appropriate specimens, (throat swabs, anal swabs, etc. including acute and convalescent blood specimens) are collected from persons with illness and their associates together with clinical and epidemiologic data from the following patient material:

(a) Children two months to four years of age admitted as in- or out-patients to D.C. General Hospital and other hospitals with and without (controls) acute infectious illnesses.

t B. included

Methods and patient material (Cont.)

(b) College students admitted as in- or out-patients at the University of Maryland infirmary with and without acute infectious illness.

(c) Ill persons and their associates, particularly family contacts experiencing an outbreak of acute illness in general population of Montgomery and neighboring counties.

(d) Household and other contacts of patients in the Clinical Center having selected acute illness.

(e) Children in an institution for handicapped at Gainesville, Florida, where a severe outbreak of a herpes simplex-like disease occurred.

(f) Children 3-15 years of age in D.C. Welfare institutions at Laurel, Maryland, and at Junior Village, Washington, D.C.

(g) Healthy inmates at the Patuxent, Maryland, penal institution who volunteered to receive infectious inoculations of two new viruses.

Major Findings:

(1) Two new viruses, the Hemadsorption (HA) 1 and 2 viruses were isolated from 7 of the 155 children with acute infectious disease and none from the 105 controls in the D. C. General Hospital study. Adenoviruses were isolated from 6 of the 11 patients and from 3 of the controls. Influenza, Long and Croup Associated (CA) viruses were not isolated. Serology of ill children showed a $\geq$ 4-fold rise to HA 1 and 2 in 42%, to adenoviruses in 17%, to Asian influenza in 12%. One patient had a rise to Long virus and none to CA virus. Since only 1 of 13 controls showed serologic evidence of infection (HA 1) with a known agent whereas 61% of acutely ill patients showed antibody response to known agents, there is evidence that known viral agents account for a significantly large proportion of illnesses bringing children to the hospital. On the other hand, a significant proportion of acute infectious diseases remains of unknown etiology requiring further study. In the small numbers involved, no characteristic clinical syndrome could be shown to be significantly associated with a specific infectious agent, but rather each of the viruses was associated with the broad clinical spectrum of acute undifferentiated respiratory disease of childhood.

(2) Household contacts and other associates of persons admitted to the Clinical Center with aseptic meningitis caused by ECHO 9 virus were studied and a very high proportion were found to have evidence

of infection as indicated by illness or ECHO 9 virus isolation. This confirmed the known rapid intrafamilial spread of this infection and again demonstrated that infection may produce no, mild, or severe clinical manifestations.

(3) The study of the herpes (simplex)-like outbreak among handicapped children of the Gainseville, Florida, institution is still in progress. More than 50% of some 200 patients in two cottages developed a herpes-like illness of a severe nature with more than 10 deaths occurring. Herpes simplex virus has been isolated from two of the three patients having vesicular lesions at the time of collection of specimens.

(4) Volunteers of the Patuxent Institution were challenged with HA 2 virus and a clinical syndrome resembling the common cold was produced. Later, another group of volunteers was challenged with HA 1 virus and again a clinical syndrome resembling the common cold followed, however, several controls also developed a common cold-like illness (but without the concomitant AB rise to HA 1 virus). Further studies are contemplated.

(5) The study of Asian influenza outbreaks among children of the D. C. Welfare Institutions showed that the epidemics which occurred in October spread slowly throughout both institutions, whereas the February, 1958, outbreak spread very rapidly throughout all cottages. This may reflect seasonal influences. The few children who were present throughout both outbreaks were substantially immune to attack during the second outbreak.

Significance to the Program of the Institute

This project complements studies of many laboratory and clinical units of the Institute along lines of mutual clinical, epidemiological and laboratory interest. Epidemiological investigations are essential to establish which microbial agents are the cause of acute illness, to define nature of the illness, and to direct research to find methods for their control.

Proposed Course of Project

It is planned to continue the project, however, study methods will be altered from time to time so that efforts are directed along the most promising lines of approach to attain the ultimate objectives.

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Part B. Honors, Awards, and Publications

Publications:

Chanock, R.M., Parrott, R.H., Cook, K., Andrews, B.E., Bell, J.A., Reichelderfer, T.E., Kapikian, A.Z., Mastrota, F.M., and Huebner, R.J.

"Newly Recognized Myxoviruses from Children with Respiratory Diseases"

New Eng. J. of Med., Vol. 258: pp. 207-213 (January 30) 1958

Cramblett, H.G., Rosen, L., Parrott, R.H., Bell, J.A., Huebner, R.J., and McCullough, N.B.

"Respiratory Illness in Six Infants infected with a Newly Recognized Echo Virus"

Pediatrics, Vol. 21, No. 2, February, 1958

Chanock, R.M., Parrott, R.H., Bell, J.A., Rowe, W.P., and Huebner, R.J.

"New Viruses Observed in Children with Respiratory Diseases"

Public Health Reports, Vol. 73, No. 3, March 1958

Reichelderfer, T.E., Chanock, R.M., Craighead, J.E., Huebner, R.J., Turner, H.C., James, W., Ward, T.G.

"Infection of Human Volunteers with Type 2 Hemadsorption Virus"

Science, Oct. 3, 1958, Vol. 128, No. 3327, pages 779-780

Honors: None

Awards: None

1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

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Part A.

Project Title: Epidemiologic Studies of Microbiologic Experience in a Nursery Population

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis M. Mastrota, Dr. Leon Rosen

Cooperating Units: Virus and Rickettsial Section, LID, (Dr. Rowe, Dr. Chanock, Dr. Huebner)
Medical and Physiological Bacterial Section, LID (Dr. Cole), Naval Medical Research Institute, (Dr. Floy

Man Years (calendar year 1958):
Total: 10 Man Years
Professional: 4 Man Years
Other: 6 Man Years

Project Description:

A. Basic Objectives

This is a long term, very intensive, and fundamental study of the illness and microbial experiences of nursery children at Junior Village, a District of Coumbia Welfare Institution. The basic objective is to delineate the multitude of host-parasite-disease-relationships as they occur naturally in this group and as they can be altered by chemo prophylaxis and new vaccines. One of the chief interim objectives is the development of epidemiologic, clinical and laboratory tools and methods for delineating the above. A concerted effort is being made to find new microbiologic agents which cause disease, methods of identification of these agents, modes of spread and methods for prevention and treatment of acute illnesses, particularly respiratory illnesses. The study is designed to shed light on the nature and scope of studies which should be pursued in further search for methods of control of acute infectious diseases particularly, the large mass of acute respiratory diseases including the common cold.

Methods employed and patient material:

The study population is located in Southwest Washington, D.C. The mean daily population varies from 50 to 70 white and negro children six to 35 months of age who are in residence in Eisenhower Cottage for domiciliary care. The mean duration of residence is approximately

Part B included Yes

Methods employed and patient material (Cont.)

16 weeks. Children with illnesses are studied either in their domicile or the infirmary at Junior Village, or the Clinical Center at N.I.H., or at the D. C. General Hospital, depending upon the severity of illnesses and study interest. Random samples of study children are given penicillin prophylaxis, other samples receive various vaccine products, e.g., adenovirus vaccine, influenza vaccine, measles vaccine, Coxsackie vaccine and other new and promising products.

A full-time pediatrician, three nurses, and four nurses' assistants maintain constant medical surveillance of the children, record rectal temperatures twice every day, collect specimens for laboratory study and prepare clinical records on each of the 50 to 70 children every day, regardless of whether or not they are ill. Selected patients are sent to the Clinical Center for more intensive clinical observation. Both throat and anal specimens for virus and bacteriological study are collected on admission, on discharge, and once weekly from every child, with additional specimens at time of illness and when otherwise indicated. Blood specimens are collected on admission, on discharge, six weeks after admission, every three months during residence, and under special circumstances.

Approximately 4,000 specimens per month are collected and studied in the five cooperating laboratories. The following laboratory units do both routine and special studies: Bacteriologic studies, of the oral pharynx are carried out by Dr. Cole's unit; enteric bacteriology studies are conducted by Commander Floyd of the Naval Medical Research Center who works in collaboration with Dr. Edwards of CDC, Atlanta, Georgia, who assists with identification of enteric pathologic coli; virological study of throat and anal specimens for adenovirus and the Polio, Echo, Coxsackie group are carried out by Dr. Rosen in the epidemiological laboratory unit. Special virological studies are being conducted by many groups, e.g., Dr. Chanock, Dr. Rowe and others.

Major Findings:

By serologic testing of bloods collected less than one week after first admission, it is evident that children in the general population groups of this area are commonly infected with adenovirus types 1, 2, 3, and 5, at a very young age. For example, by one year of age, 15% to 25% have been infected with types 3 and 5, and 40% to 50% infected with types 1 and 2 adenovirus prior to admission. By three to five years of age, some 40% have been infected with types 3 and 5, and 60% to 80% with types 1 and 2. After admission to Junior Village, and during their short period of stay, the average of some 56 weeks' observations indicated that 30% of the children had an onset of illness characterized by temperatures of 100.6°F. or greater every week and an average of 13% per week have onset of illness with fever $\geq$ 101.6°F.

Adenovirus types 1, 2, 3, and 5, are commonly prevalent in spite of prior immunity status and types 6, 9, 10, 13, and 16, have also been recovered. Recurrent outbreaks of types 2 and 3 polio virus infections have occurred with very high incidence and no paralytic cases. All children are routinely vaccinated. Outbreaks of Coxsackie B virus have been extensive and routine tests for Coxsackie A are not complete. Echo viruses have been extremely prevalent, particularly types 7, 8, 11, 12, and 14, and newly discovered JV 1 virus (Echo 20).

It is astounding that an average of some 40% of the children have a polio, Echo, or Coxsackie virus isolation each week. Many tissue culture cytopathic agents have been found which are not yet classified and some of these appear to be newly discovered viruses. Never before has such an intensive study been conducted on the microbial and illness experience of young children and the study presents heretofore nonexistent opportunities for observing fundamental principles of epidemiology. The host-parasite-disease-relationships in this study group having such a high prevalence of illness and viruses is being analyzed. In addition, streptococcal, shigella, and hemophilus infections are prevalent from time to time.

From preliminary analyses, it is evident that in more than 3,000 virus isolations from some 1,500 children, only a comparatively few are obviously incriminated by the methods used, as the cause of acute illness. Of the 40 odd different types of viruses isolated, adenovirus types 1, 2, 3, and 5, influenza virus, HA type 1 virus, polio type 2 virus, and Coxsackie B, type 3, virus are undoubtedly inducing a substantial number of acute febrile respiratory illnesses.

Significance to Program of the Institute:

It is significant that long term Junior Village studies of host-parasite-disease-relationships are being carried out on the bulk of diseases which commonly cause misery and absenteeism from schools and industry. The studies are of such a nature that they are not likely to be conducted by other than a government group. In this search for fundamental epidemiologic knowledge, the studies are progressing meticulously and fairly satisfactorily to the investigators.

Proposed Course of Project:

In July 1958, after three years of study, it was thought profitable to discontinue the routine testing of all specimens collected so as to enable analyses of results and to allow time for serologic studies and further efforts toward the identification of viruses isolated. The collection of specimens and the clinical epidemiologic observations are continuing, but the routine testing has been drastically reduced. Random samples of the children are being given different new polyvalent vaccines now at hand for preliminary test and other

new polyvalent vaccines are in process of preparation so that by the end of 1958, a multipolyvalent vaccine prepared from viruses obviously producing illness will be administered to some 40% of the children to evaluate its practical effectiveness in preventing acute illness. It is planned to continue the study for some two years or as long as it continues to appear profitable.

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Part B. Honors, Awards, and Publications

Publications:

Rowe, W. P., Hartley, J. W., Cramblett, H. G., Mastrota, F. M.

"Detection of Human Salivary Gland Virus in the Mouth and Urine of Children"

Am. J. Hyg., 1958, Vol. 67: 57-65

Rosen, L., Johnson, J.H., Huebner, R.J., and Bell, J.A.

"Observations on a Newly Recognized Echo Virus and a Description of an Outbreak in a Nursery"

Am. J. of Hyg., Vol. 67: 300-310 (May 1958)

Rosen, L., Bell, J.A., and Huebner, R.J.

"A longitudinal study of enteroviral infections in young children"

To be published in the Proceedings of the Sixth International Congress of Tropical Medicine and Malaria. Lisbon, Portugal

Rosen, L., Bell, J.A., and Huebner, R.J.

"Enterovirus infections of children in a Washington, D. C., Welfare Institution"

To be published as a chapter in the book entitled: "Viral Infections in Infancy and Childhood", New York Academy of Medicine, Dr. H. Rose, Editor

Honors: None

Awards: None

Serial No. NIAID - 82
1 Infectious Diseases
2 Epidemiology Section
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on Enteroviruses and Other Viruses Found in the Human Alimentary Tract.

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. Joseph A Bell, Dr. Frank M Mastrota, Dr. Francis R. Abinanti, and Dr. Robert J. Huebner

Cooperating Units: D. C Welfare Department

Man Years (calendar year 1958)
Total : 5.5
Professional: 1 5
Other : 4.0

Project Description:

Objectives: To determine the role in human biology of the enteroviruses and other viruses found in the alimentary tract of man.

Methods Employed: The principal population group under study is an orphanage nursery in the District of Columbia, whose average population is approximately 60 children between the ages of 6 months and 3 years. The turnover of this population is relatively rapid, and as a result, there is a great deal of illness throughout the year. (See Project No. NIAID - 81, Dr. Bell's description of Jr Village).

Part B included: Yes / X / No /___/

Rectal temperatures of each child are obtained twice a day, and the entire group is observed and provided medical care by a full-time pediatrician.

Throat and anal swabs are obtained routinely once a week from all the children, and additional specimens are obtained when indicated Serum specimens are obtained on admission and on discharge and at various intervals between

The throat and anal specimens are tested in var ous tissue culture systems, and where indicated, in animals The sera are used for antibody studies

Finally, laboratory data are correlated with clinical, bacteriological, and epidemiologic information in an effort to determine virus-disease relationships

In addition, selected outbreaks in other populations of illness suspected to be due to the viruses under study areeinvesti-gated to provide kinds of data not available from the study of the limited age-group present in the nursery group

Cooperative studies are also carried out to determine if viruses similar to human enteroviruses which occur in domestic animals are capable of infecting man and if human alimentary tract viruses can infect domestic animals

Major Findings: The typing of enteroviruses isolated from the nursery population (4000 to 5000 isolates) during the three years from July 1955 to July 1958 was largely completed. It was found that among enteroviruses cytopathogenic for rhesus kidney cultures, at least 21 different types were present, 4 of which are previously unknown types. Most of the 21 different viruses caused extensive epidemics of infection in the nursery Studies are now in progress on the serology and clinical correlations of these infections

In addition to the enteroviruses cytopathogenic for rhesus kidney tissue cultures, at least 4 different types of Coxsackie A viruses were identified in the nursery even though testing for viruses of this type in suckling mice was very limited.

A large number of isolates of viruses which apparently belong in the enterovirus group but which are not cytopathogenic for monkey kidney cultures or pathogenic for suckling mice were isolated in the nursery. These viruses are cytopathogenic only

for human tissue culture and at least two different types occurred.

Three different serotypes belonging to the ECHO 10 family of viruses were isolated from the nursery population. Although originally classified as an ECHO virus, ECHO 10 is now generally recognized to be a representative of a new family of viruses unrelated to other human virus families. One of the three serotypes was previously unknown before its isolation in this study Three major epidemics of infection with these viruses have occurred in the nursery and these represnet essentially the only data known to be available on the characteristics of infection with these viruses in man.

At least 13 different types of adenoviruses have been encountered among the nursery children and at least 4 of these were previously unknown types Of particular interest is the finding that many of the lesser known types of adenoviruses occur primarily in the lower alimentary tract and only rarely can be recovered from the throat

Significance to the Program of the Institute: This project represents an unique effort to acquire a more complete understanding of the virologic experiences and the disease consequences of such experiences in young children. In addition, it is an extremely fruitful source of previously unknown viral agents of man No less than 11 new alimentary tract viruses have already been recovered from this population and this represents more than 15 percent of the total number of such viruses known to exist at the present time.

Proposed Course of Project: The study will be continued with emphasis on the serologic and clinical aspects of the infections which have occurred In addition, studies of these viruses in other epidemiologic situations will be undertaken to broaden the knowledge of the biology of these viruses.

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Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Rosen, L. Observations on the epidemiology of dengue in Panama. Amer. J. Hyg., 68: 45-58, 1958.

Rosen, L. Experimental infection of New World monkeys with dengue and yellow fever viruses. Am J. Trop Med & Hyg., 7: 406-410, 1958

Rosen, L. Dengue antibodies in residents of the Society Islands, French Oceania. Amer. J. Trop Med. & Hyg., 7: 403-405, 1958.

Rosen, L., Johnson, J H., Huebner, R.J , and Bell, J A. Observations on a newly recognized ECHO virus and a description of an outbreak in a nursery. Amer. J. Hyg., 67: 300-310, 1958.

Cramblett, H.G., Rosen, L., Parrott, R H , Bell, J A , Huebner, R.J , and McCullough, N.B. Respiratory illness in six infants infected with a newly recognized ECHO virus. Ped., 21: 168-177, 1958

Rosen, L , Bell, J A , and Huebner, R J A longitudinal study of enteroviral infections in young children. Proc. VI Int. Cong on Trop Med and Malaria. (In press)

Rosen, L , Bell, J.A., and Huebner, R J Enterovirus infections of children in a Washington, D C. Welfare Institution Chapter in book entitled: "Viral Infections in Infancy and Childhood", N Y Acad Med , (In press), edited by Rose, H

Honors and Awards relating to this project:

None.

Serial No. NIAID - 83
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of Simple and Rapid Techniques for the Identification of Viruses and Viral Antibodies.

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. Robert Huebner and Mr. Horace Turner

Cooperating Units: None

Man Years (calendar year 1958)
Total: 2-6/12
Professional: 6/12
Other: 2.0

Project Description:

Objectives: To devise simple, rapid, and generally applicable techniques for identifying newly isolated viruses and classifying them within proper generic categories. To devise simple and rapid tests for detecting viral antibodies.

Methods Employed: Attempts to characterize and identify viral agents simply and rapidly are made, using tissue culture techniques, animal inoculation, the complement-fixation reaction, and the hemagglutinating properties of viruses. Neutralization tests are presently used for the identification of the more than 70 separate viruses falling within the enterovirus and the adenovirus families. Attempts will be made to replace these cumbersome, expensive, and time-consuming tests by cheaper, easier, and more rapid techniques. Similar procedures will be used to achieve the same ends in regard to the identification of viral antibodies.

Part B included: Yes [X] No [ ]

Major Findings:

Complement-Fixation. A CF typing procedure for the entire enterovirus family has been perfected to the point where more than ninety percent of untyped enteroviruses can be identified by this procedure. Many of the viruses which are untypable by this technique probably are representatives of new serotypes.

Hemagglutination.

Enteroviruses. With the discovery (by another laboratory) of the hemagglutinating properties of certain enteroviruses, intensive investigations were undertaken in this field and methods have been worked out for typing hemagglutinating enteroviruses by an H-I technique. For these types, this procedure is even simpler and more rapid (one hour) than the complement-fixation procedure mentioned above.

Procedures have also been developed for performing H-I tests with human and animal sera with each of the hemagglutinating enteroviruses. This involved the determination of methods of removing inhibitors and agglutinins from sera, and of the species of erythrocyte and physical and chemical conditions for the virus. Hemagglutinins were discovered for several enterovirus types for which this property was previously unknown.

Adenoviruses. The hemagglutinating properties of human adenoviruses were discovered during the year in this laboratory. Methods for typing adenoviruses by an H-I procedure were developed and more than 200 isolates, (representing more than 15 types) were typed by this procedure. Conditions were also worked out for performing H-I tests with human and animal sera. An investigation was started of different biologic subgroups in the adenovirus family, based on hemagglutination and other properties of the viruses.

ECHO 10. A hemagglutination technique was developed for typing members of the ECHO 10 family of viruses (which apparently are unrelated to other enteroviruses) and the use of this procedure was instrumental in the discovery, in this laboratory, that this family is composed of at least three distinct serotypes. An H-I technique for use with animal and human sera was developed and has been used extensively for studying several epidemics of infection with these viruses.

Tissue Culture: It was found during the year that many Coxsackie A viruses, and other enteroviruses which are not cytopathogenic for monkey kidney cells could be successfully grown in human kidney cultures. This makes possible the more effective study of these viruses by simplifying isolation and serologic procedures.

Significance to the Program of the Institute: The development of the procedures referred to above has greatly facilitated the study of the biology of the various viruses and has resulted in an almost incalculable saving in time and money. For example, by using an H-1 technique it was possible to identify as a strain of ECHO 11, in one hour, the supposedly new virus called "U-virus", which had been isolated in Sweden and studied extensively there (with eight publications). The identification of this virus had been previously missed in typing by conventional neutralization tests because of antigenic differences from the prototype strain of ECHO 11. It was easily identified by the H-1 technique in which antigenic variation is less important, following which relationships in neutralization tests were also demonstrated.

Proposed Course of Project: It is proposed to continue these investigations in order to further extend and simplify the techniques.

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Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Halonen, P., Rosen, L., and Huebner, R.J.: Typing of ECHO viruses by a complement fixation technique. Proc. Soc. Exper. Biol. and Med., 98: 105-106, 1958.

Rosen, L.: Hemagglutination by adenoviruses. Virology, 5: 574-577, 1958.

Halonen, P., Huebner, R.J., and Turner, H.C.: Preparation of ECHO complement-fixing antigens in monkey kidney tissue culture and their purification by fluorocarbon. Proc. Soc. Exper. Biol. and Med., 97: 530-535, 1958.

Honors and Awards relating to this Project:

None

Serial No. NIAID - 84
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
Calendar Year 1958

Part A.

Project Title: Studies on Rubella and Infectious Mononucleosis.

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. R. M. Chanock, Dr. K. Johnson, Dr. A. Z. Kapikian.

Cooperating Units: None

Man Years (calendar year 1958)
Total: 12/12
Professional: 6/12
Other: 6/12

Project Description:

Objectives: To isolate and study the causative virus of rubella. To determine the etiology of and develop a serologic test for infectious mononucleosis.

Methods Employed: A large epidemic of typical rubella in a girls' school was studied and specimens were collected under ideal conditions for virus isolation attempts. These specimens will be studied in various kinds of tissue culture, in animals, and by the fluorescent antibody technique. Human volunteer studies are also planned.

Sporadic cases of infectious mononucleosis will be studied in attempts to isolate the etiologic agent and to determine the origin of the heterophile antibody response.

Part B included: Yes [ ] No [X]

Major Findings: Isolation attempts for the rubella virus have been negative to date. An improved method of performing heterophile antibody determinations has been found.

Significance to the Program of the Institute: In view of the importance of rubella as a cause of congenital defects, it is highly desirable that the causative virus of this disease be isolated so that a suitable vaccine can be prepared.

Infectious mononucleosis is an important cause of morbidity in young adults and here again, it would be highly desirable to identify the etiology of this illness so that appropriate preventive measures might be undertaken.

Proposed Course of Project: It is proposed to continue studies on this project using such new techniques as may be discovered in the study of these and other infections.

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1. Infectious Dis
2. Basic Studies
3. Bethesda, Maryl

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Rabies prophylaxis

Principal Investigator: Dr. Karl Habel

Other Investigators: None

Cooperating Units: Expert Committee on Rabies, World Health Organization

Man Years:

Total	1 4/12
Professional	2/12
Other	1 2/12

Project Description:

Objectives:

To improve methods of rabies prophylaxis in man and to reduce non-specific reactions caused by vaccine.

Methods Employed:

Through the WHO Expert Committee on Rabies, various prophylactic schedules with several types of vaccine either alone or combined with antiserum are given to normal humans and the patients bled at intervals. Quantitative determinations of antibody levels are done. The same schedules are tested in animals where immunity to challenge can be performed.

Major Findings:

Sera from a large human study in Nigeria are currently being tested. Certain reduced schedules of vaccine prophylaxis have been shown in animals to be less efficient than the standard procedure.

Part B included Yes ☐ No ☒

Significance to the Program of the Institute:

These studies have direct practical application to public health practice in the field and have resulted in improvement of rabies prophylaxis in man.

Proposed Course of the Project:

Field studies with WHO will continue. Further investigations in the laboratory will be aimed at methods of eliminating the paralytic factor from vaccines.

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1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

Part A.

Project Title: Basic Studies of Virus-host Cell Relationships.

Principal Investigator: Dr. Karl Habel

Other Investigators: Dr. K. K. Takamoto, Rosalie Silverberg and Nancy Gregg.

Cooperating Units: None

Man Years:

Total	3 7/12
Professional	2 1/12
Other	1 6/12

Project Description:

Objectives:

To determine mechanism of and factors influencing attachment, invasion, multiplication and release of viruses from susceptible and resistant cells.

Methods Employed:

Various strains of tissue culture cells either suspended or on glass are exposed to viruses and at different stages of infection the cells are studied for viability and virus content. All quantitation is by plaque methods.

Major Findings:

Viruses adsorb efficiently to susceptible cells but not to resistant cells. After adsorption they can be washed off resistant cells more easily. Eclipse of virus to a non-infective state occurs

Part B included Yes [x] No [ ]

rapidly after adsorption. Attempts to find the mechanism responsible for eclipse have thus far been unsuccessful.

Significance to the Program of the Institute:

Elucidation of factors and mechanisms involved in the early stages of virus infection of the cell will advance our knowledge concerning basic principles involved in nucleic acid and protein synthesis by animal cells. It may well be that this information when eventually placed on a specific biochemical basis will be the most promising approach to chemotherapy of virus infections.

Proposed Course of the Project:

Continued investigation of the eclipse phase of virus infection will include the attempt to show the cellular component responsible for this phenomenon. The cellular site for production of various viral antigens and the cellular aspects of natural resistance will also be studied.

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1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Takemoto, K. K. and K. Habel. Virus cell relationships in a carrier culture of HeLa cells and Coxsackie A9 virus. Accepted for publication in Virology.

Serial No. NIAID-87
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Epidemiological and laboratory investigations of infectious diarrhea of infancy.

Principal Investigator: Rosalie J. Silverberg

Other Investigators: Dr. Karl Habel

Cooperating Units: Dr. Ralph S. Paffenbarger, Robert A. Taft Environmental Health Center, Cincinnati, Ohio.

Man Years:

Total	1 5/12
Professional	5/12
Other	1

Project Description:

Objectives:

To determine if causative viruses might be demonstrated for epidemic diarrheal diseases of children.

Methods Employed:

In collaboration with Dr. Ralph Paffenbarger of the Taft Environmental Health Center, the children in a small orphanage have been followed for one year for infectious diseases. Our laboratory has checked rectal and throat swabs for viruses by tissue culture methods while enteric bacteriological studies were done by the laboratory in Cincinnati.

Major Findings:

222 virus isolations were made from the specimens collected biweekly for one year. 10 were identified as Coxsackie B-3, 93 as

Part B included Yes ☐ No ☒

Echo 7, 11 as Echo 14, and 69 as adenovirus. 39 isolations were made of a virus, as yet unidentified, whose properties differ from the known enteric agents. Isolations of this agent were confined to a single period of 12 weeks, and the distribution suggests spread within the population, with prolonged fecal excretion.

No etiological role could be demonstrated for any of the enteric viruses; no viruses were isolated during an outbreak of diarrhea in the population. Data for the adenoviruses have not been analyzed as yet for possible connection with respiratory illnesses in the group.

Significance to the Program of the Institute:

Identification of an etiologic agent in infant and childhood diarrhea, obscure in most outbreaks at present, is the first step toward effective control. Data on asymptomatic infections with enteric and respiratory viruses, particularly in the very young age group under study, is of epidemiological interest.

Proposed Course of the Project:

Typing of the adenoviruses and identification of single group of enteric agents remains to be done. No further specimens will be collected.

Serial No. NIAID-88
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cell growth inhibiting substances.

Principal Investigator: Dr. John W. Hornibrook

Other Investigators: Dr. Karl Habel and Dr. Hilton B. Levy

Cooperating Units: None

Man Years:

Total	2 9/12
Professional	1 6/12
Other	1 3/12

Project Description:

Objectives:

To find and investigate substances and mechanisms which will inhibit the growth of mammalian cells.

Methods Employed:

Isolation from tissues of substances which are tested by tissue culture methods for growth inhibition.

Major Findings:

A crude fraction has been obtained from guinea pig tissues which will inhibit growth of human and mouse cells in tissue cultures. Crude casein has been found to have the same effect.

An incidental finding is that a thermolabile substance in human, and to a lesser extent in other animal serums, will kill normal and malignant mouse cells. This is apparently not complement.

Part B included Yes [x] No [ ]

Significance to the Program of the Institute:

An elucidation of the mechanism of action of these substances would be of interest to those using tissue cultures and indirectly to those in cancer research.

The effect of fresh human serum on mouse tissue may be of importance in the titration of antiserum in these animals.

Proposed Course of the Project:

Attempts will be continued to investigate the mechanism of action of these substances in a more purified state.

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1 Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

Part B: Honors, Awards & Publications

Habel, Karl; John W. Hornibrook and Nancy C. Gregg. Cytotoxic Effects of Antisera Against Tissue Culture Grown Human Epithelial Cells. Annals of the N. Y. Acad. of Sci. 69, Art. 4, 801-803, Dec. 16, 1957. Reported last year as "In Press."

Habel, Karl; J. W. Hornibrook, N. C. Gregg, R. J. Silverberg, and K. K. Takemoto. The Effect of Anticellular Sera on Virus Multiplication in Tissue Culture. Virology 5, No. 1, Feb. 1958. Reported last year as "In Press."

Serial No. NIAID-89
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Mutation in Animal Viruses

Principal Investigator: Dr. K. K. Takemoto

Other Investigators: Dr. Karl Habel and Nancy Gregg

Cooperating Units: None

Man years:

Total	1 11/12
Professional	11/12
Other	1

Project Description:

Objectives:

To isolate, purify, and characterize virus mutants.

Methods Employed:

Plaque techniques with various virus-cell systems are employed to isolate and purify viral mutants. Mutants which form plaques morphologically distinct from parental types can easily be isolated and studied further by this technique. Selective factors responsible for mutation are also evaluated.

Major Findings:

A substance in horse serum has been observed to inhibit plaque formation of type 1 poliovirus in monkey kidney cells. When horse serum at a concentration of 5% is added to the overlay medium, there is a

Part B included Yes ☐ No ☒

delay in appearance of plaques and the size of plaques is reduced markedly. However, 5 to 10% of the particles give rise to large plaques resistant to the "inhibitor." The large and small plaque formers appear to be stable characteristics. The serum inhibitor is a heat stable (56° for 30 minutes), non-dialyzable factor associated with the gamma globulin fraction of the serum. Neutralization studies indicate that it does not behave like antibody.

In a study of EMC virus and L cells, 2 types of particles capable of producing different and distinct plaques have been observed. One type forms large, round plaques while the other forms small, irregular plaques. Reversions from one type to the other occur at a fairly frequent rate.

Significance to the Program of the Institute:

Studies on variations and mutations of viruses lead to a better understanding of the nature of viruses.

Proposed Course of the Project:

Mutants derived thus far will be further examined for other properties. Stable mutants when obtained may be utilized in studies of such phenomena as interference and genetic interaction.

Serial No. NIAID-90
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Host-Parasite Relations

Principal Investigator: Dr. Victor H. Haas

Other Investigators: None

Cooperating Units: Dr. Hilton B. Levy (NIAID/LID), Dr. George Briggs (NIAMD), Dr. Michael Potter (NCI), Dr. Edwin M. Lerner (NIAMD).

Man Years:

Total	2
Professional	1
Other	1

Project Description:

Objectives:

To study the effects of antimetabolites and dietary deficiencies related to nucleic acid and protein synthesis, upon the response of mice, rabbits, and guinea pigs to the virus of lymphocytic choriomeningitis, upon the reaction of mice to certain transmissible tumors, and upon the course of the virus infection and tumor progress in mice bearing virus-infected tumors. The broad objective is to further our understanding of how nucleic acid synthesis and protein synthesis determine the outcome of infection with the virus studied and how they affect the fate of tumor-bearing mice.

Methods Employed:

Mice of suitable strains are given antimetabolites or maintained on synthetic diets deficient in certain vitamins, amino acids, or

Part B included Yes [x] No [ ]

proteins. The effects of antimetabolites and deficient diets are indicated by changes in the ability of mice to survive infection with lymphocytic choriomeningitis virus and by the prolonged survival of the virus in such mice. Transmissible ascites tumors are passaged with and without virus (LCM) contamination, in mice immune or non-immune to the virus. Rabbits and guinea pigs are injected with virus to provide immune serum. Histopathological studies are made from time to time.

Major Findings:

The most interesting observation this year has been that amethopterin makes it possible for LCM virus to survive in previously immunized tumor-bearing mice, for at least as long as 9 passage generations. Untreated immune mice, whether bearing tumors or not, rapidly eliminate virus, and become virus-free (so far as our tests can determine) during the first passage. Amethopterin-treated immune mice, not bearing tumors, also free themselves of demonstrable virus. Only the immune mice which are both amethopterin-treated and tumor-bearing seem incapable of eliminating virus.

As a part of these studies on LCM in tumor-bearing mice (all of this work is being done with Dr. Potter), it has appeared that purposeful contamination of tumors with virus is easily accomplished. Virus and tumor pass together from mouse to mouse apparently indefinitely. Though the virus seems to exert a destructive effect on the tumor, as previously reported, careful attention to time of passage enables one to avoid losing the tumor. Resistance of the tumor to amethopterin can be produced regardless of whether virus is present or not.

Amethopterin has enabled tumors to develop in several strains of mice normally resistant to them. Such mice die of their tumors and the malignant cells can be passed back successfully to the normally susceptible strain.

These and earlier observations indicate that amethopterin must significantly depress the immune response of mice both to LCM and to the tumors employed. Why should depression of immune reaction and sparing of life from an otherwise fatal infection with LCM develop simultaneously? One would expect that such an effect on immunity would cause even a more severe infection. And what is the meaning of this combination of lessened immunity-lessened fatality if it can be applied to mouse tumors, as it now seems it can?

Last year's experiments indicated that histidine deficiency caused mice to respond to LCM in much the same way as folic acid deficiency (or amethopterin administration). Repetition of these experiments with more mice (in cooperation with Dr. Briggs) shows that histidine deficient mice outlive those on a non-deficient diet, but the effect, though

definite, has been less striking than with folic acid deficiency. A single experiment with tryptophane deficient diets (72 mice) gave results comparable with those in histidine-deficient mice. It now begins to appear that interference with protein metabolism may be significant in ICM infection in the same way as interference with nucleic acid metabolism, but perhaps to a lesser degree, within the limits of experimental demonstration.

Repeated experiments have shown that reserpine protects mice against ICM, as compared with untreated ones, though all the treated mice have died. These results are similar to those reported by others for certain mouse tumors. The dose of reserpine needed is so high that the action here should not be attributed to the release of serotonin. Perhaps it is caused by vitamin or amino acid deprivation arising from the stupor produced.

The histopathological studies made by Dr. Lerner have been completed and published. They show that amethopterin suppresses cellular response to ICM in mice, but does not eliminate it, since many mice appear to develop delayed inflammatory reactions.

Significance of the Program to the Institute:

We do not know how a virus rearranges the metabolism of a cell in such a way that the cell produces the necessary nucleic acids and proteins which form new virus particles. Whatever happens, cellular nucleic acid synthesis and protein synthesis are significantly altered. And if we regard infection in the broad sense as penetration of a cell by an antigen, then we can even believe that the discoveries to be made in the infected cell will be applicable to cancer.

Proposed Course of the Project:

During the coming year I hope, in cooperation with Dr. Levy, to try to locate the site of ICM replication in infected cells, using the fluorescent antibody technique. More dietary deficiency experiments (with Dr. Briggs) may help to clarify the nature of the pathways involved in response to ICM. It should also be possible to determine the effect of deficiencies or antimetabolites on demonstrable antibody production. For some time I have wanted to see whether ICM carried with tumor transfers could acquire any transducing qualities, and perhaps I can try this (with Dr. Potter) during the year.

Related to this possibility is one of recombination among various strains of the virus, with or without reference to the tumor, and this too will be part of the program as time permits. Dr. Baron (DBS) and I have cooperated on some preliminary experiments. These studies alone may not show why the phenomena observed with antimetabolites have occurred, but they should, along with ramifications that will doubtless develop, clarify some of the causative features.

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2. Basic Studies
3. Bethesda, Maryland

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Part B: Honors, Awards & Publications

Levy, Hilton B., and Victor H. Haas. Alteration of the Course of Lymphocytic Choriomeningitis in Mice by Certain Antimetabolites. Virology 5: 401-407 (1958). Reported last year as "In Press."

Lerner, Edwin M., and Victor H. Haas. Histopathology of Lymphocytic Choriomeningitis in Mice Spared by Amethopterin. Proc. Soc. Exptl. Biol. & Med. 98: 395-399 (1958).

Briggs, G. M., D. J. Ingle and V. H. Haas. Effect of Dietary Factors on Folic Acid Deficiency in the Mouse. Fedn. Proc. 17, #1849 (1958).

Methods Employed:

The experimental approach to this problem involved an attempt to infect uniformly a population of host cells with the virus under study, and then to examine the virus-host system by suitable laboratory techniques for several metabolic activities. As in the past, physicochemical methods such as manometry, radioisotope techniques, electrophoresis, chromatography and spectrophotometry will play an important role in our experimental procedures. In addition to the tissue culture work as in past years, this year has seen the extensive use of animals infected with leukemia-inducing virus. There is reason to believe that a large percentage of the spleen cells become involved in this disease.

Major Findings:

Intravenous inoculation of mice with Friend's leukemia virus leads to the beginning of increased spleen size in about 4 or 5 days. However, infection causes an increased uptake of P^{32} by these spleens about 2 days before pathology or spleen growth is manifest. Various lines of evidence indicate that this increased P^{32} uptake is not due just to growth of the tissue. X-ray or Cortisone can induce a temporary (10-14 day) remission of the disease as indicated by decrease in spleen size, but the accelerated P^{32} uptake is still seen. Comparison was made with the following diseases, all of which show spleen enlargement and/or pathology: Lymphocytic choriomeningitis, histoplasma capsulatum, 3 transplanted leukemias, a spontaneous leukemia, and another virus-induced leukemia (Gross's). Of these only the spontaneous and other virus induced leukemias showed increased P^{32} uptake under our test conditions.

Biochemical fractionation of the radioactive infected and control spleens revealed that the metabolic sites that were affected varied with the length of infection time, and were to some extent dependent on whether or not growth had occurred. The earliest metabolic alteration with regard to phosphorus metabolism occurred in the RNA and lipid of the microsomal and the "soluble" fraction. This was found when no spleen enlargement was seen and when pathological alterations were barely detectable, if at all.

Fluorescent antibody studies done in our laboratory revealed that the viral antigen is cytoplasmic, with particularly high concentrations around the nuclear and cell membranes.

It should be recalled that the microsomal and soluble fractions of the cell are the ones on which current biochemical thinking places most of the responsibility for protein synthesis and for at least some

RNA synthesis, such syntheses can be carried on in vitro or in isolated cell fractions. It is presumed by biochemists that the soluble material contains the RNA, many different molecules of which serve as templates for the many different proteins of the cell. By an interchange between the soluble fraction, which activates and incorporates amino acids and microsome particles, protein would be built up. These are the subcellular elements which are activated by infection. A reasonable working hypothesis to account for our results is that the viral nucleic acid, on entering the cell combines, either as a whole or in part, with the soluble RNA, thus disturbing the cell equilibrium. As a result, new templates are set up and new viral protein is made in the microsomal fraction.

This hypothesis for the beginning stages of replication of at least this virus is consistent with our findings with fluorescent antibody, where viral antigen is found concentrated around nuclear membranes, because in a cell actively producing protein, there is a concentration of microsomal material around the nuclear membrane.

Significance to the Program of the Institute:

The recent developments of this project point a way to the possible explanation, at a metabolic level, of the mechanisms of reproduction of at least one virus, in this case a mammalian virus causing cell proliferation. Possible implications in the field of cancer, and the relationship between viruses and cancer, make this project of value.

Proposed Course of Project:

Since the RNA of the soluble fraction appears implicated in the early stages of virus replication, further work on this material is contemplated. We plan to subfractionate this RNA to see if we can find a subfraction that structurally or metabolically appears peculiar to the infected tissue. Using such material as a template, an attempt will be made to cause replication of virus protein in a cell-free system, suitably fortified with necessary enzymes, etc.

Serial No. NIAID-91
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, Wallace P.; Janet W. Hartley, Bernard Roizman, and Hilton B. Levy. Characterization of a Factor Formed in the Course of Adenovirus Infection of Tissue Cultures Causing Detachment of Cells from Glass. J. of Exptl. Med. 108: 713-729, Nov. 1, 1958.

Levy, Hilton B. and Isadore Brodsky. Metabolic Alteration in Leukemia Induced by a Virus. Nature 182: 1379-1380, Nov. 15, 1958.

Levy, Hilton B. and Isadore Brodsky. The Effect of a Leukemia Virus on Phosphorous Uptake by Mouse Spleen. Cancer Research. In Press.

Levy, Hilton B. and Isadore Brodsky. Some Biochemical Effects of Infection with Friend's Leukemia Virus. Ann. N. Y. Acad. Science. In press.

Levy, Hilton B. and Victor H. Haas. Alteration of the Course of Lymphocytic Choriomeningitis in Mice by Certain Antimetabolites. Virology 5: 401-407, 1958.

Levy, Hilton B., Wallace P. Rowe, LeRoy F. Snellbaker and Janet W. Hartley. Biochemical Changes in HeLa Cells Associated with Infection by Type 2 Adenovirus. Proc. Soc. for Exptl. Biol. & Med. 96: 732-738, 1957.

Serial No. NIAID-92
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Simplified Method of Preparation of Gamma Globulin for Biological Studies.

Principal Investigator: Dr. Hilton B. Levy

Other Investigators: None

Cooperating Units: Dr. Herbert Sober, NCI

Man Years:

Total	6/12
Professional	2/12
Other	4/12

Project Description:

Objectives:

To develop an extremely simple method of preparation of gamma globulin from serum or plasma.

Methods Employed:

This project was for the development of a method, and, therefore, Methods and Major Findings are incorporated together in this report.

Major Findings:

It was found that when serum that had been dialyzed vs. 0.0175M PO_4 pH 6.3, was poured into suitable DEAE cellulose columns, the gamma globulin was not absorbed, while the other serum proteins were. Therefore, the serum gamma globulin could be recovered simply by pouring the

Part B included Yes ☐ No ☒

dialyzed serum into the column and washing the column with a little more of the same buffer. The great bulk of the antibodies to a number of diseases was found, as expected in this gamma globulin fraction, though some antibodies were found in some high molecular weight gamma globulin (macroglobulins) that remained on the column.

Significance to the Program of the Institute:

In the fields of immunology and virology, the availability of a simple method for gamma globulin preparation will enable investigators to eliminate some of the confusion that arises from the use of more complex protein mixtures. This should be particularly valuable in fluorescent antibody studies and in eliminating nonspecific inhibitors in various tests such as complement fixation or H. I.

Proposed Course of the Project:

The initial purpose of the project has been achieved, and a manuscript embodying these results along with some others is being prepared. Possible further simplifications may be investigated, but most of the future activity will be in applications of the procedure.

Serial No. NIAID-93
1. Infectious Diseases
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of Abnormal Macromolecular Constitutents in Virus Infected Tissues

Principal Investigator: Dr. Hilton B. Levy

Other Investigators: Drs. I. Brodsky, K. Ballard and W. P. Rowe

Cooperating Units: Dr. K. Ballard, DBS

Man Years:

Total	6/12
Professional	2/12
Other	4/12

Project Description:

Objectives:

To examine virus infected tissues, particularly tissue cultures for the presence of abnormal macromolecular constituents, to isolate any found, to characterize them chemically, to determine what biological activity they have and how they are related to the process of viral replication.

Methods Employed:

Normal and virus infected tissue cultures were extracted and the extracts examined by means of analytical ultracentrifugation for the possible presence of abnormal macromolecular components. Some of the extracts were fractionated chemically and examined individually for biological activity and for spectrophotometric characteristics.

Part B included Yes [ ] No [x]

Major Findings:

Dr. Ballard, the investigator who ran the analytical ultracentrifuge, left the NIH in the spring of 1958 and the work was terminated then. However, some interesting results were obtained: infection of HeLa cells with adenovirus types 1, 5, and 7, and with Coxsackie virus type B_3 led to the production of two components, or possibly three, that either were not present in normal tissue culture, or were present in an extremely small concentration. Infection with type 3 poliovirus did not lead to the production of these components. The S values of the two components are approximately 65 and 145 corresponding to molecular weights of under 100,000. Thus, identity with virus particles is excluded. Attempts to characterize the peaks chemically or biologically have been too fragmentary to warrant discussion.

Significance to the Program of the Institute:

As with my other projects, here also we hope to gain information about the mode of replication of animal viruses and their effect on the host cell. The identification of these abnormal constituents, either as abnormal host constituents or incomplete pieces of virus (if indeed there is any difference between these two) should throw light on the alteration in synthetic processes induced by infection with this virus. Positive identification of these components with some biological property such as complement fixing antigen would have general interest. Examination of other virus host cell preparations should prove useful in seeing whether some biological grouping of viruses can be made on the basis of abnormal macromolecules produced.

Proposed Course of the Project:

A manuscript embodying the results obtained is being prepared. If, upon preparation, it appears sufficiently complete to warrant publication, it will be submitted for publication. The future program depends on availability of time and personnel.

Serial No. NIAMD-94
1. Infectious D...
2. Basic Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigations of interference with enzymatic functioning of mitochondria as a mechanism for carcinogenesis.

Principal Investigator: Dr. Marie L. Hesselbach

Other Investigators: Dr. Roger W. O'Gara, NCI

Cooperating Units: Laboratory of Pathology & Histochemistry, NIAMD
Laboratory of Pathology, NCI

Man Years:

Total	1 6/12
Professional	1
Other	6/12

Project Description:

Objectives:

To determine whether interference with the functioning of mitochondrial enzymes leads to neoplasia. Specifically, it is desired to find a single dye which: 1) is adsorbed by mitochondria; 2) effects the functioning of mitochondrial enzymes, and 3) induces neoplasia. Then, perhaps, a connection between these two functions of a single agent can be demonstrated.

Methods Employed:

Tumors have been produced by repeated injection of two triphenylmethane dyes (Fast Green, FCF, and Light Green, SF, yellowish). The metabolism of these tumors is being compared with that of other tumors, and of normal rat tissues. The effects of the two dyes on the functioning of mitochondrial enzymes of normal rat tissues are under study.

Part B included: Yes ☐ No ☒

Efforts are being made to find a method by which significant amounts of the dye, Janus green B, which is known to affect mitochondrial enzymes, can be injected into rats or mice. When such a method is at hand, this dye will be examined for cancerogenic activity.

Major Findings:

Control rats routinely injected with distilled water did not develop any neoplasms, whereas those concurrently receiving dye injections produced a very high percentage of tumors. Animals receiving a single injection of dye or distilled water have not developed tumors during 17 months of observation.

The original, induced, tumors appeared somewhat earlier in the Fast Green-treated animals, especially in those which initially received 6% dye. This is probably due to the fact that a larger total amount of this dye could be given before acute toxic effects limited its administration.

Cytological variation within single tumors is as great as that seen in the whole group. Structure varied from areas with spindle cells and considerable collagen to highly cellular areas.

Four tumor lines, originating from these dye-induced tumors, are being maintained.

It is an interesting side observation that the two tumor lines originating from Light Green-induced tumors sometimes produce marked changes in the testes (atrophy of seminiferous tubules), ovaries (hypertrophy, excessive luteinization, hemorrhage), and adrenal glands (hypertrophy, hemorrhage). One of these lines also often metastasizes, by embolus, to the lungs.

Preliminary studies of the metabolism of the induced and transplanted tumors show that they have a very low rate of glycolysis, while respiration has not been demonstrable.

Studies of the effects of the dyes on the metabolism of normal rat tissues have shown that, at the concentration tested, Light Green affected a number of enzymes that Fast Green does not, while a few other enzymes are affected by both dyes.

Significance to the Program of the Institute:

Both viruses (exogenous) and altered subcellular particles (endogenous) have been implicated as the cause of cancer. This study is an effort to determine the relation of mitochondria to these possible oncogenic agents.

Proposed Course of the Project:

Metabolic analysis of the tumors, with special reference to their mitochondria, will be completed.

Further studies of the biochemical and biophysical effects of Light Green and Fast Green will be undertaken.

It will again be attempted to produce tumors with Janus green B.

Further studies of the enzyme complement of mitochondria will be made. When more is known on this subject, it will be easier to discover the whole role of these organelles in normal and neoplastic life processes.

Attempts will be made to assist Dr. du Buy in transferring mitochondria from tumor cells to normal cells, to see whether the latter cells then become neoplastic. This would also clarify the role of mitochondria in neoplasis.

Attempts will also be made to help Dr. du Buy in his studies of the effects of carcinogenic agents on parts of the cell other than the mitochondria.

The two triphenylmethane dyes will be provided to Miss Jane Shoemare so she can study their staining and other morphological reactions with living mitochondria.

Serial No. NIAID-95
1. Infectious Diseases
2. Basic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Characterization of Mitochondria

Principal Investigator: Jane Showacre

Other Investigators: Dr. H. du Buy

Cooperating Units: Dr. W. C. Alford, Chemistry Section, NIAID, and Dr. D. L. Vivian, Laboratory of Cancer Pharmacology, NCI.

Man Years:

Total	9/12
Professional	6/12
Other	3/12

Project Description:

Objectives:

To discover the biochemical nature of the specificity of mitochondrial subvital staining with Janus green B and other dyes.

Methods Employed:

The specific staining of mitochondria with Janus green B was shown to depend on specific sorption of the dye on mitochondrial loci, and on the electron-transferring properties of Janus green B in the presence of enzyme-flavin complexes. In order to study the electron-transferring property of the dye separately, supernatants from fractionated cells were investigated. Reduction of the dye, lactic acid formation and oxygen uptake were measured manometrically and spectroscopically. For comparison a second dye, phenazinium metho sulfate was extensively studied.

Part B included: Yes ☐ No ☒

Major Findings:

A number of dehydrogenases, present in liver mitochondria, were found in liver supernatants functionally connected with flavoprotein. Their activity could be measured by oxygen uptake in the presence of Janus green B and the appropriate substrate. The same dehydrogenases, present on brain mitochondria, were found in brain supernatants without functional flavoprotein. Their activity could be measured by oxygen uptake in the presence of phenazinium metho sulfate and the appropriate substrate. This dye oxidizes coenzyme I and thus detects coenzyme I requiring dehydrogenases without a flavoprotein intermediary. The conditions under which phenazine can be used have been defined.

Significance to the Program of the Institute:

Differential use of the two dyes can elucidate the complexity of enzymes in biological fluids, and supply an index for mitochondrial stability.

Proposed Course of Project:

Completed, except for application to other metabolic problems.

Serial No. NIAID-96
1. Infectious Diseases
2. Basic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cytopathogenic effect in single cells in tissue culture.

Principal Investigator: Jane L. Showacre

Cooperating Units: None

Man years:

Total	9/12
Professional	6/12
Other	3/12

Project Description:

Objectives:

To study the morphological and metabolic effects of virus infection of individual virus infected cells in tissue culture.

Methods Employed:

Monkey kidney and established human and other cell lines are grown on cover slips, then placed in a special tissue culture chamber. Under direct phase and dark field microscopy the virus inoculum is introduced. Subvital staining is also used to determine changes in dehydrogenase activity. Cine- and still photomicrographic records are made.

Major Findings:

A special tissue culture chamber with motor-driven liquid flow regulation has been adapted for phase and dark field microscopy. The penetration and intracellular multiplication of microscopically visible pathogens such as toxoplasma is under study. The organisms appear to grow

Part B included: Yes ☐ No ☒

in the cells with little visible effect on mitochondria or other cytoplasmic components in early stages of infection. "L" cells have so far proven the most favorable test material for microscopic studies in the flow chamber.

Significance to the Program of the Institute:

Morphological or vital staining evidence of early specific effects of virus invasion of a cell may indicate the locus of virus effect.

Proposed Course of the Project:

Further study of morphological changes of subcellular elements following the introduction of pathogenic bacteria and viruses.

Serial No. NIAID-97
1. Infectious Dis...
2. Basic Studies Se...
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biology of mitochondria and its relation to endogenous and viral diseases

Principal Investigator: Dr. H. G. du Buy

Other Investigators: Dr. M. L. Hasselbach, J. L. Showacre and M. A. Steigner

Cooperating Units: Dr. W. C. Alford, Chemistry Section, NIAMD

Man Years:

Total	1 3/12
Professional	9/12
Other	6/12

Project Description:

Objectives:

Biological definition of normal versus pathogenic or virus-altered mitochondria.

Methods Employed:

The methods encompass the application of the cytochemical findings reported under project No. NIAID-95. They are also concerned with increasing the efficiency of extracting media for maintaining functional integrity of isolated mitochondria, as measured metabolically. The results are applied in tissue culture for long term maintenance of isolated mitochondria.

Part B included Yes [x] No [ ]

Major Findings:

A survey of mitochondria from various organs was expanded, in order to find mitochondria which maintained their metabolic function for periods longer than 6-8 hours. Mitochondria prepared from guinea pig intestines remain fully active for extended periods. Their role in multiplication of appropriate viruses is being studied.

Significance to the Program of the Institute:

If maintenance of the metabolic function of mitochondria in vitro could be accomplished for periods longer than 6-8 hours, the importance of these elements for the cell could be further shown, and their role in virus multiplication determined.

Proposed Course of Project:

Further experiments on the role of mitochondria in protein and nucleic acid metabolism will be done. Attempts at in vitro cultivation will be continued, especially with subcellular fractions obtained from cells in tissue culture. Modification of mitochondria by chemical means will be studied.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

du Buy, H. G. and M. L. Hesselbach. Glucose Utilization by Washed Mitochondria of Normal and Neoplastic Mouse Tissues. J. of Natl. Cancer Inst. 20: 403-408, Feb. 1958.

du Buy, H. G. Mitochondria in Brain as the Sites of its Metabolic Activity. Neurology 8: 69-71, April 1958.

Serial No. NIAMD-56
1. Infectious Di
2. Basic Studies
3. Bethesda, Maryl

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Comparison of the properties of crude and crystallized Coxsackie A-10 virus.

Principal Investigator: Dr. E. G. du Buy

Other investigators: Mary Steigner

Cooperating Units: Dr. W. C. Alford (Laboratory of Chemical Pharmacology) and Dr. F. S. Brackett (Laboratory of Physical Biology), NIAMD.

Man Years:

Total	9/12
Professional	3/12
other	6/12

Project Description:

Objectives:

To obtain preparations of purified crystalline virus and compare its properties with that of crude Coxsackie A-10 virus.

Methods Employed:

Coxsackie A-10 virus of suckling mouse origin is purified and concentrated by chemical and ultracentrifugal means. Purified virus is analyzed for protein and nucleic acid content.

The antigenic activity at various stages of purification is compared with that of crude preparations, using neutralization, agar-diffusion and other techniques. The virus nucleoprotein is chemically compared with the appropriate nucleoprotein of the host (sucking mouse muscle).

Part B included: Yes ☐ No ☒

Major Findings:

The antigenic activity of purified virus diluted to the same titer as the crude suspension is similar to that of the crude virus when injected by the routine method in adult male mice. One ml. of a 1/100 dilution of antiserum obtained by the use of either antigen will neutralize 10^6 to 10^8 infective doses of either crude or purified virus. There seems to be a linear relationship between antigen and antibody at all concentrations studied, indicating that no tissue antibodies are involved.

Significance to the Program of the Institute:

Definitive information concerning physical and chemical properties of purified animal viruses is very meager. Such information is essential to the better understanding of the mechanisms of virus infection

Proposed Course of the Project:

Further immunological analysis of the virus at various stages of purification is being carried out. Preliminary studies on the comparison of the nucleoprotein of the host tissue and the virus are in progress.

Serial No. NIAID - 99
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Growth and Metabolism of Rickettsiae

Principal Investigator: Elizabeth B. Jackson

Other Investigators: Hope E. Hopps and Joseph E. Smadel

Cooperating Units: None

Man Years (Calendar year 1958):
Total : 4 1/3
Professional: 2 1/3
Other : 2

Project Description:

Objectives: The objectives are: 1) the elucidation of the nutritional and metabolic requirements for the proliferation of the rickettsiae and, conversely, a study of some inhibitors of rickettsial multiplication; 2) accumulation of information which may lead to eradication of the carrier state in persons convalescent from typhus and typhoid fevers through studies on tissue cultures infected with

Part B included: Yes [X] No [ ]

rickettsiae or Salmonella typhosa and subjected to various therapeutic regimens; 3) laboratory evaluation of current commercial epidemic typhus vaccines and development of improvements in the vaccine and its assay.

Methods Employed: The ordinary procedures used in a research laboratory engaged in studies on rickettsial and other microbial agents were employed.

Major Findings: 1) Rickettsial Growth. Observations summarized in last year's report emphasized the extreme sensitivity of the rickettsiae to the metabolic status of their host cells; interference with normal host cell activity either through nutritional depletion or the presence of antimetabolites and enzyme inhibitors markedly decreased rickettsial growth and survival. Recent experiments have shown that temperature of incubation also influences the ability of the cells to support rickettsial proliferation. Thus, multiplication of the microorganisms occurred in cells held in complex medium incubated at 25°C, 35°C and 40°C but maximal growth was appreciably delayed at the lowest and highest temperatures. On the other hand, in cells kept alive in simple balanced salt solution at the 3 temperatures, rickettsial survival was an inverse function of temperature, being maximal at 25°C.

Exhaustive efforts to substantiate the claims of Paretsky et al regarding successful propagation of rickettsiae in an acellular medium yielded consistently negative results even when materials supplied by the Kansas workers were employed. Although several types of observations made by the Kansas group were duplicated in some of the current tests, these did not warrant an interpretation of proliferation of rickettsiae. Instead they indicated: a) persistence of viability of a minute number of the original R. burneti inoculated into the medium, and b) small changes in the total protein nitrogen in the culture which were within the limits of variability of the analytical method. Details of the work were reported to the Commission on Rickettsial Diseases, AFEB, in the spring of 1958. 2) Carrier State. The annual report of 1956 reported successful "curing" of tissue cultures which had been infected with R. tsutsugamushi and treated with chloramphenicol. These studies have been amplified and are described in two publications listed under Part B. Most of the current efforts along such lines have been devoted to attempts to "cure" tissue cultures infected with Salmonella typhosa. In these experiments, the design of which was

based on the host cell-rickettsia model, cultures of Earle's L929 mouse fibroblast cell were infected with the Ty-2 strain of S. typhosa at a ratio of about 50 bacteria to one L cell; after an incubation period of about one hour, the cells were washed 3 times to remove as many extracellular organisms as possible and then incubated at 35°C. Since the bacteria grew rapidly in the rich medium employed for cultivation of the tissue cells, streptomycin (20 ug/ml) was added to all the infected cultures to depress extracellular growth; this concentration of antibiotic did not markedly inhibit intracellular growth of the organisms. After 24 hours when it was ascertained by microscopic examination of stained preparations that intracellular multiplication had occurred, the infected cells were washed and resuspended in fresh medium containing only the antibiotic or antibiotic combinations under test.

In vitro sensitivity tests carried out in both bacteriological medium and in the tissue culture medium gave, with one exception, comparable values and indicated that growth of the organism was completely inhibited by 6.2 units/ml of penicillin, 3.12 ug/ml of streptomycin and 6.2 ug/ml of chloramphenicol. Growth was completely inhibited by 2.0 ug/ml of chlortetracycline when tested in nutrient broth and by 25 ug/ml when tested in tissue culture medium. In the infected cell system none of these antibiotics when employed singly at 3 - 10 times these concentrations was capable of freeing the cultures of bacteria. However, successful "cures" were obtained in two experiments in which combinations of antibiotics were employed, i.e., one with chloramphenicol and streptomycin and one with chloramphenicol, streptomycin and penicillin. In each case it was necessary to treat for at least 3 weeks to eliminate all of the bacteria; moreover, the proportion of successful "cures" was low, being 2 in 13 attempts. While apparent "cures" were observed in two instances with a combination of penicillin and chloramphenicol, S. typhosa was eventually recovered from each of these cultures. Experimental failures were due in large part to the emergence of S. typhosa variants, resistant to one or more of the antibiotics employed. The present observations emphasize the difficulty of inhibiting intracellular growth even when clinically effective levels of antibiotic are present. Since the organism was highly sensitive to the antibiotics when tested in vitro, one is led to suspect that: a) in the intracellular position the bacteria are not subjected to an effective concentration of antibiotic either because of poor penetration or of sequestration of intracellular antibiotic, or b) the bacteria may be in an altered metabolic state approaching that of the resting organism which is less susceptible to antibiotic action than is the rapidly growing bacterium.

3) Typhus Vaccine. During the course of studies designed for another purpose Dr. Winston H. Price of Johns Hopkins immunized a group of 150 children, aged 7-12, with 2.0 ml of commercial epidemic typhus vaccine given over a 7-month period. Serial specimens of sera collected before, during and after the course of inoculation and samples of the vaccines employed were made available for examination by this Unit. The lots of vaccine on reassay met the potency standards for commercial typhus vaccine. After completion of vaccinations approximately three-fourths of Price's entire group developed complement fixing antibodies while neutralizing antibodies (antitoxin) developed in 29 of the 30 persons (95%) whose sera were tested. Although the antibody responses evoked by these current vaccines may be considered as fairly satisfactory, they were somewhat lower than those obtained under comparable circumstances during the early 1940's with the typhus vaccines produced at that time.

Significance to the Program of the Institute: The basic studies of this group concerning rickettsial behavior in tissue cultures provides information on a facet of intracellular parasitism all but ignored by others, i.e., the paradoxical situation of microbial agents which are capable of carrying out a wide variety of metabolic activities in vitro yet are completely dependent on their host cells for growth and reproduction. The rickettsia-host cell model provides an excellent tool for studying not only nutritional and metabolic interrelationships but problems of the chemotherapy of infected cells as well.

Studies on rickettsial vaccines pursued on a limited but continuing basis provide an opportunity for introducing new techniques and ideas into the preparation of materials which might possess greater immunogenicity and stability.

Proposed Course of the Project: Investigations concerning the role of the host cell and its environment on the growth of rickettsiae in tissue culture cells will be continued. In attempting to gain further insight into the problem of latent infections, emphasis will be placed on a study of the mechanism by which rickettsial infectivity is rapidly lost when these organisms are present in malnourished cells.

Experiments concerning the elimination of Salmonella typhosa from infected tissues will be continued but with some de-emphasis unless more striking therapeutic effects can be obtained with several other antimicrobial substances now under consideration.

Studies on epidemic typhus vaccine will be continued with the objectives of a) developing a purified, concentrated, highly immunogenic preparation of R. prowazeki, stable in the lyophilized state for a number of years, to be used as one component in a polyvalent vaccine; b) developing a more sensitive and reproducible potency assay for epidemic typhus vaccine than that currently employed.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hopps, H.E., Jackson, E.B., Danauskas, J.X., and Smadel, J.E. Study on the growth of rickettsiae. III. Influence of extracellular environment on the growth of Rickettsia tsutsugamushi in tissue culture cells. J. Immunol. in press (to appear early 1959).

Hopps, H.E , Jackson, E.B., Danauskas, J.X., and Smadel, J.E. Study on the growth of rickettsiae. IV. Effect of chloramphenicol and several metabolic inhibitors on the multiplication of Rickettsia tsutsugamushi in tissue culture cells. J. Immunol. in press (to appear early 1959).

Woodward, T.E., and Smadel, J.E. Rickettsial infections, Chapters 159-167. In Harrison, T.R. (ed.): Principles of Internal Medicine, 3rd edit. New York, McGraw-Hill Book Co. Inc., 1958, pp. 1020-1038.

Smadel, J.E. Hemorrhagic fever, Chapter 191. In Harrison, T.R. (ed.): Principles of Internal Medicine, 3rd edit. New York, McGraw-Hill Book Co. Inc., 1958, pp. 1099-1101.

Honors and Awards relating to this project:

None

Serial No. NIAID - 100
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Serologic epidemiology of numerous and prevalent virus infections.

Principal Investigator: Dr. R. J. Huebner

Other Investigators: Dr. W.P. Rowe, Dr. L. Rosen, Dr. R.M.Chanock, Dr. F. R. Abinanti, and Mr. H.C. Turner.

Cooperating Units: NINDB, Collaborative study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood (Dr. Richard Masland).

Man Years (calendar year 1958)

Total:	5/12
Professional:	1/12
Other:	4/12

Project Description:

Objectives: To apply newly developed viral serologic methods to a broad and integrated study of the roles of numerous newly recognized viruses (70 to 80) in the high morbidity of acute undifferentiated respiratory diseases, chronic diseases, birth defects, and birth wastage.

Methods Employed: Simplified serologic techniques, including complement fixation tests, hemagglutination-inhibition, hemadsorption, neutralization, and tissue culture neutralization tests are now available for the diagnosis of 70 to 80 common viral diseases. Some of the tests give group responses, others, specific responses; some require additional standardization for mass testing.

Part B included. Yes [X] No [ ]

A major problem is the technical one of producing standardized and otherwise suitable antigens and virus suspensions and antiserum for such tests. This is largely a logistic problem which can be solved readily by stimulating interest in producing such materials by reputable commercial concerns. This latter problem resolves itself into the matter of financing the reagent production enterprise. The solution of this aspect of the problem has been assumed by NINDB.

Another problem, now in the process of being worked out, is the supply of well-trained viral serologists. In collaboration with NINDB, 6 technicians of various levels and experience in virus research, have been employed by LID, and are being trained on the job on research problems having to do with respiratory, enteroviral, and other common virus diseases. Within the next year, they will be integrated into the present activities of the LID serology team, and some will likely be separated into a new serologic team designed to deal with mass serologic studies not currently covered by existing serologic activities.

Human serums for viral diagnosis and retrospective analysis of viral experiences will be made available from current studies of ill children and adults with the common acute undifferentiated respiratory diseases, from epidemiological studies of special suspected virus disease problems of contemporary interest to LID, and the serial bleedings taken from mothers during the course of pregnancy and from the infants at and after birth, as outlined in the Collaborative Study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood, of NINDB.

Major Findings: See: NIAID-LID Projects Nos. 82, 83, 84, 101, 102, 103, 104, 105, 106. The subject matter of this program has previously been described in other special projects. It is now being formalized as a new project.

Significance to Program of the Institute: Serologic epidemiology of virus diseases represents the only method by which the importance of some viruses in human disease can be determined. This is particularly true of viruses that are difficult to isolate or viruses whose pathogenic activity may be difficult to determine, or whose effects may be delayed or not recognized until long after infection, as is the case of rubella and birth defects.

Collaboration with NINDB in this study will enable us to determine the value of serologic epidemiology in defining the clinical importance of 70 to 80 new viruses of man, something which was impossible to conceive of only several years ago, because of the lack of tools available at that time.

Proposed Course of the Project: See: "Methods Employed".

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1. Infectious Diseases
2. Virus & Rickettsial Diseases
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PART B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Huebner, R.J.: Illnesses due to adenoviruses. Modern Medicine, 26: 103-108, 1958.

Huebner, R.J.: Clinical importance of "new" respiratory viruses. Postgraduate Med., 23: 356-359, 1958.

Huebner, R.J.: Coxsackie and ECHO viral infections. IN: Cecil-Loeb Textbook of Medicine, 10th Edition, Russell L. Cecil, M.D., Editor, W.B. Saunders Co., Philadelphia. In press.

Huebner, R.J.: Implications of 70 newly recognized viruses in man. Proceedings of Symposium on Perspectives in Virology, Monograph of the Institute of Microbiology, Rutgers University, New Jersey. In press.

Huebner, R.J.: The adenoviruses: general properties, clinical picture, and laboratory diagnosis. Proceedings of the Sixth International Congresses on Tropical Medicine and Malaria, Lisbon, Portugal. In press.

Greenberg, M., and Huebner, R.J.: Rickettsialpox, 1958. Proceedings of the Sixth International Congresses on Tropical Medicine and Malaria, Lisbon, Portugal, In press.

Honors and Awards relating to this project:

Dr. R.J. Huebner:

Presented invited paper at Symposium on "Perspectives in Virology", sponsored by Hartz Mountain Products Corp., and Rutgers University, in memory of Dr. F.R. Beaudette.

Presented lecture at Harvard University School of Public Health, as Visiting Lecturer on Microbiology, December, 1958.

Presented lecture at Virus Institute, Bratislava, Czechoslovakia, September, 1958.

Part B continued:

Honors (continued):

Dr. R. J. Huebner:

Presented lecture at Institute of Epidemiology and Microbiology, Prague, Czechoslovakia, September, 1958.

Presented two invited papers at 6th International Congresses on Tropical Medicine and Malaria, Lisbon, Portugal, Sept. 1958.

Presented invited paper at American Public Health Assn. meetings in St. Louis, Mo., October, 1958.

Presented invited paper at Conference of State and Provincial Public Health Laboratory Directors, St. Louis, Mo., October, 1958.

Requested by WHO to participate in a meeting of a scientific group on virus diseases, with the purpose of determining how best an extension of the research activities of the WHO may be achieved. Geneva, Switzerland, November, 1958.

Serial No. NIAID - 101
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Viruses as Causes of Respiratory Illness in Infancy and Early Childhood.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. M. K. Cook, Dr. R. J. Huebner, Dr. Karl M. Johnson, Dr. A. Z. Kapikian

Cooperating Units: Dr. Robert Parrott, Children's Hospital Research Foundation, Washington, D. C.

Man Years (calendar year 1958)
Total: 50/12
Professional: 10/12
Other: 40/12

Project Description:

Objectives: 1. To search for new agents responsible for respiratory illness in infancy and childhood. 2. To study the epidemiology of viruses which have recently been associated with respiratory illness. 3. To continue the study of the natural history of certain well-established respiratory viruses, i.e., their epidemiology and contribution to the overall respiratory disease experience during infancy and childhood.

Part B included Yes [X] No [ ]

Methods Employed: Infants and children with respiratory illness and suitable controls without such illness will be studied at Children's Hospital of D.C. The severe respiratory disease syndromes such as bronchiolitis, laryngotracheobronchitis, and pneumonia will be investigated in hospitalized patients while the milder febrile respiratory disease syndromes will be studied among clinic patients and other special infant population groups.

The main emphasis will be on the use of various tissue culture techniques for virus isolation. The established methods successful in the isolation of adenoviruses, influenza, "CA", hemadsorption viruses, and respiratory syncytial virus will be utilized. For the myxoviruses, i.e., influenza, CA, and the hemadsorption viruses, the hemadsorption technique will be employed for the detection of virus in inoculated tissue cultures. Exploratory studies will attempt to apply the fluorescent antibody technique in the search for new viruses, specifically, hypothetical agents which may grow in tissue culture but which cannot be recognized by a cell destruction effect or by hemadsorption.

Viruses isolated during the course of this study will be identified by serologic means and by other biologic characteristics. Agents which cannot be typed with the available antiserums will be characterized and an effort will be made to define their role in human disease. In addition to virus isolation, the incidence of infection with known and new agents will be estimated by a serologic study employing paired serums from as many respiratory patients and control individuals as possible.

Major Findings: During the past year, two new respiratory viruses have been recovered using the hemadsorption technique combined with monkey kidney tissue culture. One hundred and thirty-four strains have been isolated from children with respiratory illness. All the isolates could be classified into one of two serologic groups - either Type 1 or Type 2 hemadsorption virus. Both agents were shown to be members of the myxovirus family and distinct from the influenza viruses.

The studies carried out in the hospitals indicated that the hemadsorption viruses were associated with respiratory illness. These agents were recovered from the oropharynx of 6% of infants and children with respiratory disease. This forms a lower limit for the estimate of illness associated with the hemadsorption viruses. Serologic study of paired serums from patients with respiratory disease indicated that 27% of such illness was associated with hemadsorption virus infection.

The most striking association of these viruses with disease was seen with acute laryngotracheobronchitis, the most serious respiratory illness in childhood. Approximately 30% of such disease was associated with Type 2 hemadsorption virus infection.

Significance to the Program of the Institute: Respiratory disease is the most frequent infectious disease affecting man. During infancy and childhood, respiratory infections occur most commonly and in their severest form. As a first step toward immunoprophylaxis, it is necessary to define the agents responsible for respiratory illness. In addition, a systematic study of the type described should contribute to a better understanding of the natural history and relative importance of certain recently described viruses ("JH", "2060", "CA", and "respiratory syncytial") in the total respiratory disease complex. The benefits of such information will not be limited to the pediatric age group, since all available evidence indicates that the newer agents associated with illness in infancy and childhood are also associated with diseases of a generally milder nature in adults.

Proposed Course of the Project: It is planned that this study will continue for a period of several years, since the available information indicates that viruses responsible for respiratory illness vary at different times and in different localities. Therefore, surveillance should continue in order to comprehend the total picture.

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Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Parrott, R.H., Cook, K., Andrews, B.E., Bell, J.A., Reichelderfer, T., Kapikian, A.Z., Mastrota, F.M., and Huebner, R.J.: Newly recognized myxoviruses from children with respiratory disease. New England J. Med., 258: 207-213, 1958.

Chanock, R.M., Parrott, R.H., Bell, J.A., Rowe, W.P., and Huebner, R.J.: New viruses observed in children with respiratory diseases. Pub. Health Repts., 73: 193-195, 1958.

Chanock, R.M., Vargosko, A., Luckey, A., Cook, M.K., Kapikian, A.Z., Reichelderfer, T., and Parrott, R.: Association of hemadsorption viruses with respiratory illness in childhood. J.A.M.A. In press.

Chanock, R.M., Parrott, R.H., Cook, M.K., and Bell, J.A.: New viral agents recovered from the respiratory tract of infants and children. Proceedings of the N.Y. Academy of Medicine Symposium on Viral Diseases of Infancy and Childhood. In press.

Honors and Awards relating to this project:

Dr. Robert M. Chanock selected as Member of Committee for Classification of Myxovirus Group of the International Nomenclature Committee, 7th International Microbiologic Congress, Stockholm, August, 1958.

Serial No. NIAID - 102
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness.

Principal Investigator: Dr. Maryjane K. Cook

Other Investigators: Dr. Robert M. Chanock, Dr. Robert J. Huebner
Dr. Karl M. Johnson

Cooperating Units: None

Man Years (calendar year 1958):
Total: 50/12
Professional: 10/12
Other: 40/12

Project Description:

Objectives: 1. To investigate the biological and antigenic properties of newly recognized viruses associated with respiratory illness. 2. To work out methods for the production of antigens to be employed in the serological epidemiology of respiratory disease. 3. To adapt certain agents to growth in tissue culture in order to facilitate serodiagnoses and epidemiologic studies.

Part B included: Yes [ ] No [X]

Methods Employed: The standard methods of virus characterization are employed. Host range in different types of tissue culture and animals, growth in the embryonated egg, size, resistance to various chemical agents, agglutination of various erythrocytes, and most important of all, antigenic analysis. Antigenic analysis includes not only tests with specific animal serums but also includes a study of reactions of the antigen in question with paired serums from persons infected with related viruses. The antigenic characterization of the newer viruses employs the neutralization, complement-fixation, hemagglutination-inhibition, and hemadsorption-inhibition techniques.

For the production of antigens for serologic investigations, intensive study is required to determine the optimum host system and the most favorable conditions for virus growth.

Fluorescent microscopy involving the use of fluorescein labelled antibody is employed for the identification of an agent suspected of an association with respiratory illness, i.e., primary atypical pneumonia (PAP) virus. According to present information, this agent grows only in embryonated eggs, does not produce observable changes in the affected cells, and can only be worked with in the laboratory with the fluorescent antibody technique. This technique will be employed to determine if virus is present in any of the various tissue culture host systems in which adaptation will be attempted.

Major Findings: The recently recovered hemadsorption viruses were classified into 2 groups - hemadsorption virus Type 1 and Type 2. The biologic properties of the 2 viruses were studied and found to be those characteristic of myxoviruses. Complement fixation and hemagglutination antigens were prepared for both viruses for use in epidemiologic surveys. Antigenic analysis using serums from immunized animals and infected persons indicated that the hemadsorption viruses types 1 and 2 were distinct from other myxoviruses. Nevertheless, the hemadsorption viruses possessed antigens in common with the other myxoviruses. The closest relationship demonstrated was between Sendai and hemadsorption virus type 2.

Work has just begun on the virus of primary atypical pneumonia utilizing fluorescent microscopy of infected chick embryo tissues as the indicator system. It has been possible to propagate the virus in chick embryos, confirming the report of Liu.

Significance of the Program for the Institute: Knowledge of the biologic and antigenic properties of the newer myxoviruses, specifically the hemadsorption viruses, is required in order to perform meaningful epidemiologic studies of the importance of these agents in respiratory disease. The problem of antigenic overlap is particularly important when one attempts to determine by serologic means the contribution of any member of the myxovirus group to disease. In studies employing single serum specimens of different age groups and from individuals residing in various localities, the proper interpretation of serologic results in the light of antigenic overlap is of great importance.

The problem of cold agglutinin positive atypical pneumonia has been in a state of confusion for at least 15 years since Eaton's original publication of the isolation of PAP virus. With the new tool of fluorescent microscopy, it should be possible to determine the role this agent plays, if any, in pneumonia. Also, the possible role of PAP virus in mild respiratory disease may prove to be of some public health importance.

Proposed Course of the Project: The problem of antigenic overlap in the myxovirus group will be studied from a more practical viewpoint. The present studies have employed *in vitro* serologic techniques. The projected studies will employ *in vivo* challenge experiments in which infection with one virus will be followed after an interval suitable to exclude interference effects with infection with a related virus. The quantitative aspects of infection, i.e., the amount of virus produced in the lung and the amount of virus required to initiate infection will be investigated.

The primary atypical pneumonia virus project will continue with attempts to grow the virus in tissue culture with the production of a cytopathogenic effect or the production of a complement fixation antigen. In addition, studies will continue in an effort to determine the role, if any, of this virus in respiratory disease.

Serial No. NIAID- 103
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Continuing Study of Adenoviruses and Other Recently Isolated Respiratory Viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. J. W. Hartley and Dr. R. J. Huebner

Cooperating Units: Epidemiology Section, LID-NIAID

Man Years (calendar year 1958)

Total:	3/12
Professional:	1/12
Other:	2/12

Project Description:

Objectives: To characterize newly isolated respiratory viruses encountered in epidemiologic studies.

Methods Employed: Isolation and serologic identification of adenoviruses in children; serologic comparison of adenovirus isolates.

Major Findings: The "Mills virus", which was recovered from children and normal HeLa cell tissue cultures on several occasions since 1954 (see Project #101), was shown to be immunologically identical with Hemadsorption virus Type 1, and represents the first isolations of this new group of agents.

Part B included Yes [X] No [ ]

Classification of adenoviruses has been continued to the extent of serving as a reference laboratory for strains isolated in various parts of the world. The most significant observation in this connection is the occurrence of adenoviruses with mixed antigens of several different combinations, and the accumulation of additional new serotypes requiring characterization.

Significance to the Program of the Institute: With the continuing importance of the respiratory infections, it is important that the etiologic agents be carefully characterized both serologically and biologically. Continuing surveillance of the antigenic structure of adenoviruses by a laboratory with prolonged experience with agents is important for following antigenic changes and possible emergence of important new types.

Proposed Course of Project: Work on respiratory viruses by this unit has been reduced and changed in emphasis. Identification of adenovirus serotypes as a reference laboratory, however, will be continued. Laboratory support will be provided to the LID-wide study of microbial infections in infants (Jr. Village study), as well as to the continuing studies of the value of adenovirus vaccines in children carried out in collaboration with the Epidemiology Section, LID.

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Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, W.P., Hartley, J.W., and Huebner, R.J.: Serotype composition of the adenovirus group. Proc. Soc. Exp. Biol. and Med., 97: 465-470, 1958.

Chanock, R.M., Parrott, R.H., Bell, J.A., Rowe, W.P., and Huebner, R.J.: New viruses observed in children with respiratory diseases. Pub. Health Repts., 73: 193-195, 1958.

Rowe, W.P., Hartley, J.W., Roizman, B., and Levy, H.B.: Characterization of a factor formed in the course of adenovirus infection of tissue cultures causing detachment of cells from glass. J. Exp. Med., 108: 713-729, 1958.

Huebner, R.J., Rowe, W.P., and Chanock, R.M.: Newly recognized respiratory tract viruses. IN "Annual Review of Microbiology", 12: 49-76, Annual Reviews, Inc., Palo Alto, Calif., 1958.

Levy, H.B., Rowe, W.P., Snellbaker, L.F., and Hartley, J.W.: Biochemical changes in HeLa cells associated with infection with type 2 adenovirus. Proc. Soc. Exper. Biol. and Med., 96: 732-738, 1957.

Merchant, R.K., Rowe, W.P., Kasel, J.A., and Utz, J.P.: Pharyngo-conjunctival fever due to type 1 adenovirus. New England J. Med., 258: 131-133, 1958.

Rowe, W.P.: Adenovirus and salivary gland virus infections in children. IN Monograph of the N. Y. Acad. Med., in press.

Gundelfinger, B.F., Hantover, M.J., Bell, J.A., Loosli, C.G., and Rowe, W.P.: Evaluation of a trivalent adenovirus vaccine for prevention of acute respiratory disease in Naval recruits. Am. J. Hyg., in press.

Serial No. NIAID - 104
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of Salivary Gland Viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. J. W. Hartley and Dr. I. Brodsky

Cooperating Units: Dr. Charles Chany, St. Vincent de Paul Hospital, Paris, France.

Man Years (calendar year 1958)

Total:	3/12
Professional:	1/12
Other:	2/12

Project Description:

Objectives: To characterize the salivary gland viruses and elucidate the epidemiology and clinical importance.

Methods Employed: Surveys of antibodies and virus excretion in humans. Studies of the biology of the salivary gland viruses of lower animals.

Major Findings: Follow-up on children known to excrete salivary gland virus has been continued, and several children are now known to be excreting virus for as long as one-and-one-half to two-and-one-half years after antibody was known to be present. Studies of the urinary sediment of virus excreters has shown no correlation between abnormal urinary sediment and presence or absence of salivary gland virus infection.

Part B included Yes [X] No [ ]

A long-term study of mouse salivary gland infection has been carried out, with the finding that virus persists in the salivary glands for longer than a year after inoculation, but that inclusion bodies are not detectable after the fourth month. Virus was present in the salivary glands in considerable titer and frequently in saliva.

In cooperation with the Children's Hospital of D.C., a strain of salivary gland virus was isolated from a case of cytomegalic inclusion body disease.

Significance to the Program of the Institute: Salivary gland virus infection is an interesting and unique infection of both humans and lower animals. Human infection is known to produce a certain number of neonatal deaths, congenital abnormalities, and secondary infections; it is possible that the virus is responsible for much more illness than is presently recognized.

Proposed Course of Project: Studies of the role of human salivary gland virus in production of congenital anomalies will be pursued as part of the birth defects study of the National Institute of Neurological Diseases and Blindness. Frequency of infection during pregnancy, as indicated by development of antibodies, will be compared in mothers with defective children as against those with normal offspring.

The possible importance of recrudescent activity of salivary gland virus in chronic diseases of adults as well as in childhood will be investigated as opportunities to do so are presented. Studies of the immunological relationships between the human salivary gland virus and a monkey strain isolated last year will be continued.

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Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rowe, W.P., Hartley, J.W., Cramblett, H.G., and Mastrota, F.M.: Detection of human salivary gland virus in the mouth and urine of children. Am. J. Hyg., 76: 57-65, 1958.

Brodsky, I., and Rowe, W.P.: Chronic mouse salivary gland virus infection. Proc. Soc. Exper. Biol. and Med., in press.

Rowe, W.P.: Adenovirus and salivary gland virus infections in children. IN Monograph of the N.Y. Acad. Med. In press. (Also listed under Project #103)

Serial No. NIAID - 105
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of Respiratory Virus Illness in a Welfare Orphanage.

Principal Investigator: Dr. R. M. Chanock

Other Investigators: Dr. M. K. Cook and Dr. R. J. Huebner

Cooperating Units: Dr. J. A. Bell, Epidemiology Section, LID-NIAID
Dr. F. M. Mastrota, " " " "
D. C. Welfare Department

Man Years (calendar year 1958)
Total: 12/12
Professional: 4/12
Other: 8/12

Project Description:

Objectives: To determine the natural history of certain newly discovered respiratory viruses in a longitudinal orphanage population.

Methods Employed: The population group under study is an orphanage nursery in the District of Columbia, whose average population is approximately 60 children between the ages of 6 months and 3 years. The turnover of children is relatively rapid and as a result, a great deal of respiratory illness occurs throughout the year.

Rectal temperatures of each child are taken twice a day. The entire group is observed and provided medical care by a full-time pediatrician.

Part B included Yes [X] No [ ]

Throat swabs are collected once a week from all children and additional specimens are taken at the time of onset of febrile illness. Serum specimens are obtained on admission and on discharge and at various intervals between.

Throat swabs are tested in monkey kidney and Chang liver tissue cultures. The serum specimens are used for antibody studies. Laboratory data are then correlated with clinical, bacteriological, and epidemiologic information in an effort to determine qualitative and quantitative virus-disease relationships.

Major Findings: From three outbreaks of respiratory illness agents were recovered by tissue culture techniques employing the hemadsorption procedure. One of the epidemics was associated with Asian influenza virus while type 1 and 2 hemadsorption viruses were recovered from the other outbreak.

The two outbreaks associated with the hemadsorption viruses yielded the most significant new information. Type 1 virus spread rapidly through the orphanage. This agent was recovered from 27 or 50% of the 54 infants and children during a 10 day interval. In each instance virus isolation was confirmed by the development of complement-fixation antibody. An additional 22% of the population from whom virus was not isolated were infected during this interval as estimated by development of antibody. Pneumonitis occurred commonly among the infected individuals. Epidemiologic analysis suggested an association between hemadsorption virus type 1 and the illnesses which occurred during this outbreak. Evidence was obtained that certain of the children had been infected previously with type 1 virus and that the current episode represented re-infection despite the presence of antibody.

Type 2 hemadsorption spread slowly through the nursery; an interval of 3 months elapsed between the first and last infection. Almost all of the infants in the nursery from whom virus was isolated developed neutralizing antibody. The epidemiologic analysis of this outbreak has not been completed.

The contrast in spread of type 1 and type 2 viruses through the nursery population is consistent with the incubation periods observed for these agents in volunteer experiments in adults. When type 1 virus was given to volunteers 2 to 3 days elapsed before onset of symptoms while this interval was 6 days for type 2 virus.

Significance to the Program of the Institute: The type 1 hemadsorption virus epidemic provided the first evidence that this agent caused respiratory illness and was capable of producing

pulmonary involvement. Important information was gained regarding the incubation period and the spectrum of illness produced by this virus.

The virus experience of infants observed over long periods should clarify the practical future steps necessary for prevention of the common respiratory illnesses of childhood.

Proposed Course of the Project: The project will be continued in an effort to gain a better understanding of the newer respiratory viruses and the technique of fluorescent miscroscopy will be used in an effort to isolate additional respiratory viruses presumably responsible for the large segment of respiratory disease which remains unexplained. Experimental vaccines for the hemadsorption viruses will be evaluated for their effectiveness in preventing infection and illness.

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Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Parrott, R.H., Cook, K., Andrews, B.E, Bell, J.A., Reichelderfer, T., Kapikian, A.Z., Mastrota, F.M., and Huebner, R.J.: Newly recognized myxoviruses from children with respiratory disease. New England J. Med., 258: 207-213, 1958.

Huebner, R.J., Rowe, W.P., and Chanock, R.M.: Newly recognized respiratory tract viruses. Annual Review of Microbiology, 12: 49-76, 1958.

Honors and Awards relating to this project:

None

Serial No. NIAID - 106
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of Respiratory Viruses and Enteroviruses in Animals and their Relationship to Human Viral Infection.

Principal Investigator: Dr. Francis R. Abinanti

Other Investigators: Dr. Robert J. Huebner, Dr. Leon Rosen

Cooperating Units: Dr. Robert Byrne, University of Maryland, Div. of Veterinary Medicine.
Dr. Robert Reisinger, Animal Disease Research Station, USDA, Beltsville, Md.

Man Years (calendar year 1958)

Total:	24/12
Professional:	12/12
Other:	12/12

Project Description:

Objectives: The objectives of this project are, 1) the isolation of common viral agents from animals; 2) the characterization of these agents, and, 3) the possible relationships and epidemiologic importance of these agents to human disease.

Methods Employed: Specimens of nasal secretions, throat swabs, and feces, from animals with suspected viral infections, are collected with sterile cotton swabs, and these are suspended in vials containing Hank's solution and antibiotics.

Part B included: Yes ☐ No ☒

Specimens for virus isolation efforts are inoculated into tissue cultures of cells originating from the homologous species, e.g., dog kidney for specimens from dogs and bovine embryonic kidney and lung for the inoculation of bovine materials, as well as the available standard tissue cultures.

Immunologic techniques are used for characterization. They are the neutralization technique using tissue cultures, complement fixation, and hemagglutination inhibition. The immunologic relationships of respiratory and enteroviruses to those of man are determined.

Major Findings: Dr. Robert Reisinger of the Animal Disease Station, USDA, isolated in tissue culture a virus from cattle with respiratory illness (Shipping Fever), designated as SF-4. Work in this laboratory showed that this virus is a strain of HA type 1 virus, first isolated in this laboratory from tissue culture systems and from children with respiratory illness. Serological surveys using complement fixation and hemagglutination inhibition tests, developed in this laboratory, provide evidence of widespread infection of cattle with this virus. We have isolated the virus on several occasions this winter from cattle with respiratory illness. Experimental infection of calves with this virus revealed a viremia during the acute febrile illness.

Infectious bovine rhinotracheitis virus has also been isolated by Dr. Reisinger and us from cattle with respiratory illness. This virus has certain characteristics of the adenovirus group but has not been shown to share common complement fixing antigen of this group.

Enteroviruses have been isolated frequently from the feces of normal adult cattle and calves. On the basis of the appearance of cytopathogenic effect, these appear to be of several different types. In the studies of bovine respiratory disease, enteroviruses have been isolated from the nasal secretions, but, so far, these have not been shown to be responsible for respiratory illness.

No viral agents were isolated from the throat swabs and feces of normal dogs and dogs having respiratory illness. Several isolations, however, were made from the uninoculated dog kidney tissue culture cells of canine infectious hepatitis virus. One such isolation was from the kidney of a new-born puppy.

Significance of Program to the Institute: The studies on the prevalence and public health significance of SF-4 virus infection in cattle is intimately associated with the studies being conducted in this laboratory on the epidemiology of the human infections with HA-1 virus. Studies are now under way to determine if cattle may not be a convenient way of assessing the potency of HA-1 vaccines to be used in human vaccine experiments.

The isolation and determination of the antigenic relationships of the enteroviruses of cattle dovetails in with studies being done in this laboratory on enterovirus infections of man.

The elucidation of the epizootiological patterns of infection in animals with these various viruses presents the epidemiologist with convenient, readily available study material.

Proposed Course of the Study: The major effort will be made to study the extent and seasonal distribution of SF-4 infection in cattle. Studies will be done to determine the presence of the virus in the blood of calves being slaughtered for veal. Milk will be sampled for the presence of the virus.

Studies will be done to determine if cattle may be artificially protected against SF-4 infection.

The cooperative project with the University of Maryland Veterinary Science Division will be continued to study outbreaks of respiratory disease in cattle.

Serial No. NIAID - 107
1 Infectious Diseases
2 Virus & Rickettsial Diseases
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of Tumor Producing Viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. Robert J. Huebner, Dr. Janet W. Hartley, Dr. Isadore Brodsky

Cooperating Units: Dr. Hilton B. Levy; Dr. Herbert Kahler, National Cancer Institute; Dr. Lloyd Law, National Cancer Institute; Dr. Ludwik Gross, U.S. Veterans Hospital, Bronx, New York; Dr. Edwin Murphy, Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine.

Man Years (calendar year 1958):
Total: 101/12
Professional: 25/12
Other: 76/12

Project Description:

Objectives: To characterize biologically, serologically and physically viruses which produce tumors in animals, and to attempt to recover similar viruses from humans.

Methods Employed: 1. Utilization of standard and new viral methods in attempts to detect and quantitate tumor viruses. 2. Epizootiologic studies of virus occurrence and serum antibodies. Attempts to isolate viruses from human tumors which exhibit similar characteristics to those of animal tumors.

Part B included: Yes [X] No [ ]

Major Findings: Most of the work has been conducted on the mouse tumor agents described by Gross, Stewart, and Eddy (Mouse Polyoma Virus) and by Friend.

A. Mouse Polyoma Virus: 1. Much of the work of Drs. Stewart, Eddy, and Gross has been confirmed. 2. It has been found that polyoma virus hemagglutinates a variety of mammalian and avian erythrocytes, acting probably on the same receptors as are responsible for myxovirus group hemagglutination. 3. A hemagglutination-inhibition procedure for detection of specific antibodies to polyoma virus has been developed and standard procedures devised suitable for large-scale application. 4. Complement-fixation tests also suitable for serologic surveys have been developed and applied. 5. Neutralization tests in tissue culture and suckling hamsters have been developed and used to demonstrate the specificity of the hemagglutination-inhibition and complement fixation tests. 6. Procedures for quantitating and detecting virus have been developed and standardized. Either tissue culture cytopathic effects reported by Eddy, et al., or production of antibodies in adult mice can be used as an index of viral infectivity (MAP test, reported from this laboratory. 7. The physical nature of the virus has been characterized. The virus is highly stable to a variety of environmental influences. Electron micrographs of purified concentrated preparations have been obtained in collaboration with Dr. Kahler 8. Epizootiologic studies in various mouse breeding colonies show that various mouse colonies differ greatly in the prevalence of polyoma virus infection, depending primarily on the intensity with which work on the virus is conducted in the laboratory and experimental animals. 9. Mice infected as newborns have prolonged chronic excretion of virus in saliva and urine and to a lesser extent in feces. 10. Mice are susceptible to infection by intranasal instillation or ingestion of virus. This finding, together with the excretion of virus in saliva and urine, probably accounts for the infection cycle observed in the laboratories working with the agent, and having high prevalence of spontaneous infection. 11. Virus can be recovered in small amount from tissues of approximately 50% of spontaneously infected mice and regularly from tumors and other organs of artificially inoculated mice. 12. Serologic tests of polyoma virus against a wide variety of known human and animal viruses have shown no evidence of antigenic relationship of polyoma virus to any other known agent. 13. Although human serums have no complement fixing antibody to polyoma virus, a high percentage of human serums have inhibitors of hemagglutination in low titer which are resistant to heating and receptor destroying enzyme. Such inhibitors have not been found in any other species except the mouse. Presence or absence of such inhibitor in human serum is not associated with neutralizing antibody for either tissue culture cytopathic effects or hamster tumors and is not correlated

with presence or absence of tumors in the human.

B. Mouse Leukemia Virus (Friend): 1. An accurate and reproducible method for quantitation of virus has been developed, using the dose response curve of spleen weight versus dose of virus. Using this assay it is possible to quantitate to 0.2 to 0.5 log accuracy and to carry out neutralization tests. 2. In collaboration with Dr. Levy in the Basic Section, accelerated uptake of radioactive phosphorous is found in spleens of mice infected with this virus. Spontaneous mouse leukemias show a similar pattern whereas transplanted mouse leukemias do not. This finding suggests that mouse leukemia produced by the Friend virus may provide a better model for spontaneous leukemia than do transplanted leukemias. 3. Fluorescent antibody staining of infected mouse spleens has shown a characteristic pattern of intense cytoplasmic staining without nuclear staining. Viral assays of subcellular fractions also indicate that virus is found predominantly in the cytoplasm.

Significance to the Program of the Institute: The recent discoveries of several viruses producing tumors in mice and of the ability of one to propagate in tissue culture have opened exciting new avenues for the study of the viral etiology of malignancies. Application of modern viral techniques and full elucidation of the properties and epidemiology of such agents by persons trained in virology and epidemiology will provide information of great value in providing guidelines for future studies of human cancer.

Proposed Course of the Project: Studies of characterization and epidemiology of the polyoma virus will be continued, with particular emphasis on elucidation of the maintenance and spread of infection in the absence of artificial environmental contamination and on studies of virus variation. Attempts will be made to develop laboratory procedures for similar study of other known tumor agents, including avian lymphomatosis, rabbit papillomatosis, the Bittner milk factor, the Gross leukemia virus, and the Friend leukemia virus. Continuing attempts will be made to recover agents from human cancer patients using concepts and procedures derived from work with the animal tumor viruses.

Serial No NIAID - 107
1 Infectious Diseases
2 Virus & Rickettsial Diseases
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Eddy, B , Rowe, W.P., Hartley, J W , Stewart, S. E., and Huebner, R.J. Hemagglutination with the S.E. polyoma virus. Virology, 6: 290-291, 1958

Rowe, W.P , Hartley, J.W., Brodsky, I., and Huebner, R J Complement fixation with the S.E. polyoma virus. Science, in press.

Rowe, W.P., Hartley, J.W., Brodsky, I., and Huebner, R.J. Observations on the spread of mouse polyoma virus infection. Nature, in press.

Levy, H.B., and Brodsky, I. A metabolic alteration in a virus induced leukemia. Nature, in press.

Levy, H.B., and Brodsky, I. Effect of a leukemia virus on P32 uptake by mouse spleen. Cancer Research, in press.

Levy, H.B., and Brodsky, I. Biochemical studies of infection with the Friend virus. Ann. N.Y. Acad. Sci., in press.

Honors and Awards relating to this project:

None

Serial No. NIAID - 108
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
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Part A.

Project Title: Studies of the Natural History of Animal Tumor Viruses as Possible Models of Human Cancer.

Principal Investigator: Dr. R. J. Huebner and Dr. W. P. Rowe

Other Investigators: Dr. J. W. Hartley, Dr. I. Brodsky, Mr. H.C. Turner.

Cooperating Units: None

Man Years (calendar year 1958)

Total:	16/12
Professional:	8/12
Other:	8/12

Project Description:

Objectives: To study the epizootiology and ecology of animal tumor viruses, utilizing newly developed, highly precise methods for quantitating the infectious disease process independent of tumor production.

Methods Employed: a) Serologic surveys for tumor virus infections occurring naturally in animal colonies. b) Utilization of epidemiologic methods to determine the extent of natural infection and mode of spread.

The viruses to be studied, probably sequentially, in this way, are listed as follows: 1) mouse polyoma; 2) Friend leukemia; 3) fowl sarcoma; 4) Gross leukemia.

Part B included: Yes [ ] No [X]

Major Findings: This is a new project based on the recent technological advances with mouse polyoma listed in NIAID Project #107. It is already apparent, using various new serologic and virus titration methods, that the spectrum of "infection" with polyoma virus in mouse colonies greatly exceeds the occurrence of clinical manifestations.

Serologic surveys of mouse breeding colonies (involving hundreds of mice) from many laboratories, have revealed exceedingly widespread natural infections with polyoma virus, yet the parotid tumors and sarcomas invariably produced by this virus when grown in tissue culture and inoculated in sucklings, are seldom observed resulting from natural infections. For additional findings providing a basis for this study, see Project # 107.

Significance to the Program of the Institute: Like paralysis in poliovirus infection, and clinical manifestations of many other common virus infections, the clinical manifestations of tumor viruses may, in nature, be uncommon or even rare, compared to almost widespread or even universal infection. Careful study of the natural history of such viral infectious disease processes are vital to understanding and eventual control. Should some hypothetical human cancer viruses behave like mouse polyoma, mouse leukemia, and the fowl leukosis complex, where widespread inapparent infection is known to occur naturally via a variety of routes, then the late, unpredictable, and often rare and unassociated tumors produced by such hypothetical viruses would appear to be non-infectious in origin. Since the methods so far used to prove the existence of animal tumor viruses (which depend on the experimental production of tumors in other animals of the same species) cannot be used in the study of human tumor viruses, the existence of such hypothetical but probable agents must be established by indirect means; possibly, by serologic epidemiology. Determinations of common denominators (both in laboratory and epidemiologic areas) characterizing tumor viruses of mice, chickens, and other animals, plus more precise information of their natural history in the proper biologic meaning of this term, will be invaluable in efforts to find and establish the importance of viruses in human cancer. Single attempts to isolate viruses from human cancer are, of course, of little value, since current studies show that numerous newly recognized prevalent human viruses could hardly fail to turn up occasionally in human tumor tissue. Their presence would have little significance in establishing their importance in producing the tumors.

Proposed Course of Project: 1) Studies of natural history of polyoma and other tumor viruses in colonies of wild mice, particularly those in large urban areas such as New York City, where viral and rickettsial agents are already known to be prevalent.

2) Application of modern viral techniques to studies of other known tumor viruses, such as the Bittner mammary agent, Gross leukemia, Friend leukemia, fowl sarcoma, the fowl leukosis complex, in order to determine similarities and differences in natural histories that may permit certain hypotheses concerning possible common denominators with hypothetical but probable human tumor viruses -- which, in turn, might provide an intelligent basis for epidemiological studies of human cancer designed to test the hypothesis that certain cancers are due to viruses.

Serial No. NIAID - 109
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of the Antigenic and Biological Properties of Animal Tumor Viruses.

Principal Investigator: Dr. Janet W. Hartley

Other Investigators: Dr. Wallace P. Rowe, Dr. Robert J. Huebner, and Dr. Isadore Brodsky

Cooperating Units: None

Man Years (calendar year 1958)
Total: 12/12
Professional: 6/12
Other: 6/12

Project Description:

Objectives: To study the antigenic and biologic properties of several tumor viruses, particularly those grown in tissue culture, in an effort to determine whether there are any possible common denominators indicating family relationships.

Methods Employed: Mouse polyoma, "Friend" leukemia, and fowl lymphomatosis viruses are currently available for antigenic and biologic analysis. Complement fixation, hemagglutination-inhibition, and neutralization techniques are used to explore the antigenic structure of the tumor viruses. Tissue culture host range, cytopathogenic effects, resistance to various physical and chemical

Part B included: Yes [X] No [ ]

effects are utilized in comparing these tumor viruses to ordinary non-tumor viruses, as well as to other tumor viruses.

Major Findings: 1. The finding that the mouse polyoma virus can agglutinate erythrocytes from a variety of mammalian and animal species has led to the development of a standard HI procedure which has been widely used in epidemiological surveys of several mouse colonies, virus quantitation studies, pathogenesis experiments in mice and hamsters, and serological differentiation from other viruses.

2. Hemagglutination by polyoma virus has been studied intensively, with particular emphasis on properties found to be held in common with the myxovirus group. Treatment of guinea pig red cells with RDE and types A and B influenza virus renders them inagglutinable by polyoma virus. Inhibitors of polyoma hemagglutination are found in normal serum and, in high titer, in ovomucin; pre-treatment of ovomucin with certain myxoviruses markedly decreases the inhibitor titer for polyoma virus. However, no enzymatic destruction of red cell receptors or ovomucin inhibitor by polyoma virus action has been demonstrated.

An apparent inhibitor of polyoma virus hemagglutination has been detected in certain serial tissue culture passage lines, red cell eluate preparations, and mouse organ suspensions. Materials negative for HA became positive at 8- to 32- fold higher dilutions following exposure to RDE or heating at 56°C. or 37°C. for 30 minutes.

3. A complement fixation test for mouse polyoma virus has been developed which has been used satisfactorily as an adjunct to the HI test, and for confirming the serological distinctness of polyoma virus from members of the myxovirus group and other viruses to which the CF test can be applied.

4. Stability studies have shown that the polyoma virus is extremely resistant to various physical and chemical treatments. Hemagglutination and infectivity titers of standard virus pools were unaffected by ether treatment, freezing and thawing, 2 months' storage at -60°C. or 4°C., heating to 60°C. for 30 minutes, treatment with phenol, zephiran, 50% alcohol, or lyophilization. Exposure to UV irradiation for 1 hour decreased infectivity but not hemagglutination titer; the virus was inactivated by heating at 70°C. for 30 minutes and by exposure to 100% alcohol and iodine-alcohol mixtures.

Significance to the Program of the Institute: By the use of standard virological techniques, it has been possible to study readily and quantitatively, the biological properties of an animal tumor virus and to develop serological tools for epidemiologic and immunologic studies. Application and modification of the techniques successful with the mouse polyoma virus may permit more detailed and

quantitative investigations of other tumor viruses and allow comparative studies in similar systems

Proposed Course of the Project: Continued efforts will be made to develop tissue culture and serological techniques applicable to the "Friend" leukemia, Bittner mammary tumor, and rabbit papilloma viruses which would permit comparison with the mouse polyoma and the fowl lymphomatosis and sarcoma viruses for which such procedures are already fully or partially developed. Studies of the hemagglutinin of polyoma virus will be continued in an effort to define the "inhibitor" phenomenon which appears to have both practical and theoretical importance.

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1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

See Part B of Dr. Rowe's Project Report NIAID - 107.

Hartley, J.W., and Rowe, W.P.: "Unmasking" of mouse polyoma virus hemagglutinin by heat. Virology, in press.

Honors and Awards relating to this project:

None

Serial No. NIAID - 110
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of a Leukemia Virus (Friend leukemia).

Principal Investigator: Dr. Isadore Brodsky

Other Investigators: Dr. Wallace P. Rowe and Dr. Janet W. Hartley

Cooperating Units: Basic Virus Section, LID-NIAID, Dr. H. B. Levy.

Man Years (calendar year 1958)
Total: 9/12
Professional: 4/12
Other: 5/12

Project Description:

Objectives: To apply contemporary and new virus techniques to the study of a leukemia virus.

Methods Employed: Serologic techniques, tissue culture, biochemical and physical fractionation of cells, and fluorescent antibody techniques.

Major Findings: By continuous passage in mice by intravenous inoculation of extracts and/or filtrates of 10% suspensions of Friend leukemia spleens, the titer of the virus preparations have been increased. At present, with high titered preparations, using spleen weight as a criterion, disease can be demonstrated grossly within 5 days after inoculation. Adequate virus titrations can now be carried out in two weeks using spleen weight as the end point. The accuracy of titration in mice compares favorably with that of extinction titrations with other viruses in tissue culture. The development of a means of quantitation for the Friend virus was essential for studies with this agent.

Part B included: Yes [X] No [ ]

Metabolic changes produced by infection with the Friend virus have been studied. Early in the course of the disease, even before spleen enlargement, the spleens of infected animals take up more radioactive phosphorus than did those in the controls. The administration of X-ray, cortisone, or chlorambucil decreased the size of the infected spleens to normal range but the uptake of P-32 remained elevated. Elevated P-32 uptake was demonstrated in two other leukemias - one a spontaneous AKR leukemia and the other a virus induced leukemia (Gross leukemia). Three transplanted leukemias studied did not show this phenomenon. Early in the course of the disease, the uptake of P-32 is limited to the RNA of the infected cells. Later, the uptake is increased in both the RNA and DNA components. Fluorescent antibody techniques using rabbit anti-Friend serum in a direct test demonstrated specific fluorescence in the cytoplasm of the infected cells. Cellular fractionation indicated that infectivity was limited to the cytoplasmic components of the cell, i.e., mitochondria and microsomes.

Significance to Program of the Institute: Studies of infection with the Friend virus are of basic interest in understanding the mechanisms of malignancy formation in animals. It adds strong support to the proponents of the virus theory of cancer. The similarity of the Friend leukemia, in terms of clinical course, response to therapeutic agents, and metabolic activity, to human leukemia is quite marked. The usefulness of this disease in screening anti-leukemic drugs has been suggested in a publication. The Friend virus will be an excellent model for future studies in the division of oncogenic and oncolytic virology in this Institute.

Proposed Course of Project: Continued attempts will be made to increase the titer of the virus so that a complement-fixation test will be obtainable. Efforts will also be continued to obtain replication of Friend virus in a tissue culture system. Determination of the virus titer before, during, and after X-ray induced remissions will be studied. This approach will also be extended in the future to cortisone and chlorambucil.

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1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Levy, Hilton, and Brodsky, Isadore: A metabolic alteration in a virus induced leukemia. Nature, 182: 1379-1380, 1958.

Levy, Hilton, and Brodsky, Isadore: Biochemical studies of infection with the Friend virus. Submitted for publication to New York Academy of Sciences, following an oral presentation of the paper.

Honors and Awards relating to this project:

NONE

NIAID
Laboratory of
Tropical Diseases

Estimated Obligations for FY 1959

Total:	$1,428,400
Direct:	1,100,000
Reimbursement:	328,400

Covering Project Reports
NIAID....120 thru 128

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Coatney, G. R.; Mickelson, O.; Young, M. D.; Burgess, R.; and Pirkle, C. I.: Chloroquine or pyrimethamine in salt as a suppressive against sporozoite-induced vivax malaria (Chesson strain). Bull. of Wld. Hlth. Org. 19: 53-67, 1958.

Coatney, G. R. and Greenberg, J.: The effect of a diet deficient in Factor 3 on the course of Plasmodium berghei infection in mice. Proc. VI Internatl. Cong. (In press).

Wright, W. H.; Dobrovolny, C. G., and Berry, E. G. Field trials by various molluscicides (chiefly sodium pentachlorophenate) for the control of aquatic intermediate hosts of human bilharziasis. Bull. of Wld. Hlth. Org. 18: 963-974, 1958.

Wright, W. H.: Introductory remarks to the Panel discussion. Proc. N. Y. Acad. of Sciences (In press).

Wright, W. H.: Progress in research and control of the helminthiases other than schistosomiasis and filariasis. Proc. VI Internatl. Cong. (In press).

Wright, W. H.; Phillips, B. P.; and Newton, W. L.: Germ-free animal research at the National Institutes of Health. Proc. VII Microbiology Cong. (In press).

Honors and Awards relating to this project:

Dr. Willard H. Wright:

Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Member of Commission on Parasitic Diseases, Armed Forces Epidemiological Board.

Member Scientific Advisory Committee of the American Foundation for Tropical Medicine.

Member of Board of Directors and of the Executive Committee of the Gorgas Memorial Institute.

Member of Expert Advisory Panel on Parasitic Diseases of the World Health Organization.

Rapporteur of Helminthic Infections Section, Division on Tropical Medicine, VI International Congresses on Tropical Medicine and Malaria, Lisbon, 1958.

Professional lecturer in Medical Zoology, School of Medicine, The George Washington University.

Official U. S. delegate to the VI International Congresses on Tropical Medicine and Malaria, Lisbon, 1958.

Recipient of the Walter Reed Medal awarded by the American Society of Tropical Medicine and Hygiene.

President-elect American Society of Tropical Medicine and Hygiene.

Dr. G. Robert Coatney:

Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Visiting Lecturer on Malaria, Georgetown University School of Medicine.

Visiting Professor, Department of Preventive Medicine and Public Health, School of Medicine, Howard University.

Rapporteur of Clinical Section, Division on Malaria, VI International Congresses on Tropical Medicine and Malaria, Lisbon, 1958.

Member of Expert Advising Panel on Malaria of the World Health Organization.

Official U. S. Delegate to the VI. International Congresses on Tropical Medicine and Malaria, Lisbon, 1958.

WHO Consultant Expert Committee on Malaria.

Awarded honorary Sc. D. by Bowling Green State University.

Serial No. NIAID-120
1. Tropical Diseases
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Administration and research planning and coordination

Principal Investigator: Willard H. Wright - until 30 June

G. Robert Coatney - since 1 July

Other Investigators: G. Robert Coatney - until 30 June

Cooperating Units: None

Man Years (calendar year 1958):

Total:	6½
Professional:	1½
Other:	5

Project Description:

This project furnishes certain technical, supervisory, and administrative services to all research projects in the Laboratory, as follows:

(a) Over-all planning of research, the coordination of research activities in the various Sections of the Laboratory, and the direction of Foreign Field Stations.

(b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

(c) Supervision over personnel, maintenance, travel, and correspondence.

(d) Requisitioning and supervision of equipment and supplies.

(e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

(f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

(g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, Government agencies including liaison activities with other branches of the Service, foreign governments, and international agencies.

(h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

Research projects are reviewed quarterly. If necessary, they are discontinued or revised in accordance with current developments and needs. Some changes have been made in the research program of the Laboratory and some activities directed into new channels. Continued emphasis is being placed on the more fundamental aspects of problems in tropical medicine, such as the metabolic requirements of parasites and the chemistry of their metabolism, the physiology of parasites in relation to their disease-producing proclivities, of relation between nutrition and schistosomiasis, the mechanism of drug action on parasites and the inhibiting effect of antimetabolites, and the carriage of viruses by parasites. Emphasis was also placed on the epidemiology and isolation of tropical insect-borne viruses, on the colonization of vectors, the tissue culture of mammalian malarias, and the use of germ-free animals in research on such problems as virulence, immunity and pathogenesis.

Part B included - Yes

Serial No. NIAID - 121-A
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Host-parasite relations in worm infections in laboratory animals

Principal Investigator: L. J. Olivier

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958)

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To study the characteristics of infection in laboratory animals; to learn how resistance to infection may be enhanced and reduced; to learn how the host defense mechanisms act to resist infection; and to study variation in resistance in host strains.

Methods Employed:

Infect animals in such a way as to produce predictable infections, treat with hormones, antigens, etc., challenge with infective worms, observe the host and the parasites, and determine by counting the lesions and the worms, and by the study of tissue sections, the degree of resistance and the host tissue reaction. Test whether Freund's adjuvant is useful in the production of resistance to worms by the injection of killed worm material.

Major Findings:

Mice made susceptible to infection by the use of cortisone do not lose their infection when cortisone is stopped. Why this occurs is not evident, since one might reason that after cortisone administration is halted the defenses of the host might be expected to kill off the worms; rats, at least, are known to develop rather solid immunity to infection.

Significance to the Program of the Institute:

Problems of resistance and immunity are basic to an understanding of host-parasite relations. Invasiveness of parasites and host reactions to parasites are important clues to an understanding of the pathogenicity of worms.

Proposed Course of the Project:

To continue study of the effects of cortisone and other hormones on worm infections. A study will also be made of the development of resistance in schistosome infections.

Part B included - No

Serial No. NIAID-121-B
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Role of helminths in the causation of cancer

Principal Investigator: L. J. Olivier

Other Investigators: E. G. Berry

Cooperating Units: 1. Pathological Anatomy Branch, NCI.-525

2. Section on Cancer Products, Laboratory of Bacterial Products, DBS.

Man Years (calendar year 1958):

Total:	1
Professional:	1/3
Other:	2/3

Project Description.

Objectives:

To test whether worm parasites can be made to induce malignant growths in the laboratory. To investigate the possibility that liver tumors of rats caused by Taenia taeniaformis infection are due to a virus.

Methods Employed:

Schistosoma haematobium. Hamsters are infected with this species in an attempt to induce malignant growths in the bladder or other organs.

Taenia taeniaformis. This parasite regularly produces liver tumors in rats. Infected rat tissue and worm material are inoculated into tissue cultures in an attempt to pass a possible cancer-producing agent.

Major Findings:

The schistosome study is still under way.

The Taenia experiments have so far not resulted in any positive findings in the attempts to isolate a possible cancer-producing agent. However, a new rat virus has been isolated and is now being studied.

Significance to the Program of the Institute:

Study of the relation of parasitic worms to malignancy should increase our knowledge of the significance of worm infections and provide new information concerning the factors which predispose individuals to cancer.

Proposed Course of the Project:

Continue long range study of the relation between these two parasites and cancer.

Part B included - No

Serial No. NIAID-121-C
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-N H
Individual Project Report
Calendar Year 1958

art A.

Project Title: Destruction of molluscs by chemical means.

Principal investigator: L. J. Olivier

Others: W. T. Hackins

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2 1/2
Professional:	1 1/2
Other:	1

Project Description:

Objectives:

To discover more effective means of killing snail vectors of schistosomiasis. To study the mode of action of chemicals used as molluscicides.

Methods Employed:

Snail vectors or their eggs are exposed to chemicals and the destructive efficacy of the chemicals is recorded. Studies are made of the action of the chemicals on the snails, such as the determination of the accumulation of the chemicals in the various snail tissues and its rate of elimination upon termination of exposure of the snail to the chemicals. Exposures are quantitated with respect to both concentration and length of exposure to the chemicals.

Major Findings:

The study has not been under way long enough to permit definite conclusions to be made. Newly laid snail eggs seem to be far more susceptible to damage by sodium pentachlorophenate than partially developed embryos or newly hatched snails. Strains of

snails also differ in the susceptibility of their eggs to this chemical. Very low concentrations of sodium pentachlorophenate appear to have a marked effect on the egg-laying habits of the snails and also upon the eggs themselves.

Significance to the Program of the Institute:

The most effective way to control schistosomiasis in many areas is by control of the intermediate host, the snail. Education of the population in proper sanitation is probably the surest method of eradication of the disease, but takes an impossibly long time to accomplish. The preliminary results from this project suggest that it may be possible to control snail populations in some endemic areas by continuous administration of low, and therefore relatively economical, concentrations of the chemical. This may be more practical in some situations than intermittent use of higher concentrations.

Proposed Course of the Project:

To continue along these lines with sodium pentachlorophenate and to investigate the effect of other chemicals at low concentrations on snail eggs and upon the adult snails, especially with regard to the effect upon their reproductive capacity and the viability of their eggs.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Barbosa, F. S. and Olivier, L.: Studies on the snail vectors of bilharziasis mansoni in North-eastern Brazil. Bull. World Health Organ. 18: 895-908, 1958.

Hairston, N. G., Hubendick, B., Watson, J. M. and Olivier, L.: An evaluation of techniques used in estimating snail populations. Bull. World Health Organ. 19: 661-672, 1958.

Olivier, L. and Barbosa, F. S.: Survival and weight loss of Australorbis glabratus kept at controlled relative humidities in the laboratory. Proc. of the 6th Intern. Congr. Trop. Med. (in press).

Honors and Awards relating to this project: None

Serial No. NIAID-12[illegible]-D
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigation of intermediate hosts and vectors of human diseases caused by worms

Principal Investigator: E. G. Berry

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1
Professional:	2/3
Other:	1/3

Project Description:

Objectives:

To investigate the distribution, life history, and ecology of snail intermediate hosts and to develop a rational and useful system of classification and means of identification.

Methods Employed:

Colonies of known snail vectors are maintained in the laboratory where they are available for intensive study. Suspected vectors are exposed to infection to evaluate their suitability as vectors in the field. Attempts are made to discover means for controlling the vectors with poisons.

Major Findings:

Continued progress has been made toward the discovery of criteria useful for the accurate identification of the snails which serve as the intermediate hosts of Schistosoma mansoni and Schistosoma haematobium. In addition, a monograph is in preparation on some of the vectors of schistosomiasis.

Significance to the Program of the Institute:

This knowledge is essential if one is to understand the epidemiology of worm infections transmitted by vectors and if one is to devise adequate control procedures.

Proposed Course of the Project:

Continue anatomical studies of snails in order to make possible their accurate identification. Study variation among snail strains with respect to susceptibility to infection with schistosomes.

Part B included - Yes

Serial No. NIAID-121-D

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this Project:

Wright, W. H., Dobrovolny, C. G., and Berry, E. G.: Field trials of various molluscicides (chiefly sodium pentachlorophenate) for the control of aquatic intermediate hosts of human bilharziasis. Bull. Wld. Hlth. Org. 18: 963-974, 1958.

Honors relating to this project: None

Serial No. NIAID-121-E
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of methods for the cultivation of parasitic helminths in vitro and the determination of the nutritional requirements of such organisms in vitro

Principal Investigator: P. P. Weinstein

Other Investigators: M. F. Jones

Cooperating Units: None

Man Years (calendar year 1958):

Total:	3 1/4
Professional:	1 1/4
Other:	2

Project Description:

Objectives:

To develop media suitable for in vitro cultivation of helminths throughout their life cycle.

Methods Employed:

Worm eggs or larvae are isolated bacteria-free, are inoculated into culture media to be tested for growth-supporting properties, and are observed for development and differentiation.

Major Findings:

Partial development of the parasitic cycle of the human hookworm, Necator americanus, has been obtained in vitro. The free-living stages were successfully reared in previous work, so that much of the life history of this parasite can now be studied in vitro under axenic conditions.

Work has continued on the improvement of the media used for rearing the free-living and parasitic stages of Nippostrongylus muris, and considerably higher yields of adult worms are now obtained. Females have deposited large numbers of eggs in culture, but these eggs have all been infertile. Therefore, the normal sequence of events concerning ovulation and copulation of this organism in vivo has been studied, as well as some physical and nutritional conditions which may bear on mating in vitro.

Significance of the Program to the Institute:

When the nutritional and physical conditions necessary for in vitro development of worms are known, basic studies may help explain clinical and pathological effects of worm infections and enable better diagnosis and treatment.

Proposed Course of the Project:

Work will be continued with different blood and tissue fractions in attempts to delineate further the growth requirements of worms in complex media, and attempts will be made to obtain development in media of known chemical composition. Factors governing mating and egg production in vivo and in vitro will be studied.

Part B included - Yes

PHS-NIH Serial No. NIAID-121-E
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinstein, Paul P.: Some projected uses for the axenic cultivation of helminths. Am. J. Trop. Med. and Hyg. 7: 1-3, 1958.

Weinstein, Paul P. and Jones, Myrna F.: Development in vitro of some parasitic nematodes of vertebrates. Ann. N. Y. Acad. Sci. (in press).

Honors relating to this project:

Dr. Weinstein was presented an Award of Honor by Brooklyn College.

Dr. Weinstein was elected to the Council of the American Society of Parasitologists

Serial No. NIAID-121 F
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Nutritional aspects of helminthiasis

Principal Investigator: W. B. DeWitt

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1 2/3
Professional:	2/3
Other:	1

Project Description:

Objectives:

To determine the role of nutritional factors in the disease processes associated with helminth infections, their effect on host susceptibility, and their possible influence on the development of resistance to reinfection.

Methods Employed:

Animals maintained on purified diets either deficient or enriched with specific components are exposed to various helminths. The influence of the diet and its consequences on the host and on the parasite are studied.

Major Findings:

A diet deficient in pantothenate inhibited the elaboration of precipitating antibodies in mice against schistosome eggs. Pantothenate deficiency, however, had no apparent effect on worm development or on the rate of egg production.

Significance of the Program to the Institute:

Debilitating helminthiases develop frequently in human populations living on a deficient diet. In order to understand the clinical problems adequately it is necessary to assess the role of the diet and of the worms in producing the debilitation. The problems of treatment, prevention, and control of helminth infections can be met more effectively if these relations are understood.

Proposed Course of the Project:

The present study will be extended to include the effects of diets deficient in vitamin B_{12}, folic acid, niacin, and the fat soluble vitamins A and D on schistosoma infections in mice. Later, the study will extend to the relation between diet and infections with other species of worms.

Part B included - yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

DeWitt, William B. and Schwarz, K.; Multiple dietary necrotic degeneration in the mouse. Experientia. 14: 28-30, 1958.

Honors and Awards relating to this project: None

Serial No. NIAID-121-G
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The development of helminths in germ-free animals

Principal Investigator: M. F. Jones

Other Investigators: P. P. Weinstein, L. J. Olivier

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1 1/12
Professional:	3/4
Other:	1/3

Project Description:

Objectives:

To determine whether the germ-free animal will support development of worm parasites and to compare the development of worms in germ-free animals with that obtained in conventional animals. To study factors of resistance to infection using germ-free hosts.

Methods Employed:

Axenic helminth inocula are prepared and inoculated via various routes into germ-free animals.

Major Findings:

Improved techniques of concentrating helminth eggs have been developed, as well as a method of determining the viability of larvae within the eggs of Hymenolepis nana. This should allow more quantitative infections in experimental animals.

Significance of the project to the Institute:

Using germ-free technique it should be possible to study cellular, nutritional, and antibody factors which may be involved in natural resistance to infections with worms. Helminths are particularly well-suited to this kind of work in germ-free animals since most of them become localized, do not multiply in the host, and can often be obtained germ-free. Germ-free animals may be a very promising means for the study of the relation between bacterial infection in the host and invasion by other organisms.

Proposed course of the project:

Suitable helminths and various germ-free hosts will be used to determine promising combinations for intensive study. Work is being done to extend some of the findings reported previously, particularly on the ability of H. nana to establish itself in the absence of intestinal bacteria.

Part B included - Yes

Serial No. NIAID-121-C

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Newton, W. L., Weinstein, Paul P., and Jones, Myrna F.: A comparison of the development of some rat and mouse helminths in germ-free and conventional guinea pigs. Ann. N. Y. Acad. Sci. (in press).

Honors and Awards relating to this project: None

Serial No. NIAID-121-M
1. Tropical Disease
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of the excretory products of helminths

Principal Investigator: W. T. Haskins

Other Investigators: L. J. Olivier

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To isolate and identify excretory products of parasitic worms. To compare species and worm groups with respect to their excretory products. To judge the influence of nitrogenous excretory products on host reactions and resistance to infection.

Methods Employed:

Chemically analyze the fluids in which larval and adult worms have been incubated under sterile conditions.

Major Findings:

The investigation of the nitrogenous excretory products of tapeworm, T. taeniaformis, larvae has shown the presence of ammonia, urea, aliphatic amines, amino acids, and a small amount of polypeptides. These findings are similar to those previously obtained from certain round worm larvae except for the presence of urea which was shown to be absent in the case of the round worms studied.

Significance to the Program of the Institute:

The understanding of the basic metabolic pathways and their end products occurring in parasitic worms is the precursor of a better knowledge concerning the clinical and pathological effects of such infections and of better means of diagnosis and treatment.

Proposed Course of the Project:

This project was temporarily suspended following completion of the investigation of the nitrogenous excretory products of T. taeniaformis larvae.

Part B included - Yes

Serial No. NIAID-121-H

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Haskins, Willard T. and Olivier, L.: Nitrogenous excretory products of Taenia taeniaformis larvae. J. Parasitol. 44: 567-573, 1958.

Honors, Awards, relating to this project: None

Serial No. NIAID-121-I
1. Tropical Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pathological physiology of worm infections

Principal Investigator: K. S Warren

Other Investigators: W. B. DeWitt

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1 1/3
Other:	2/3

Project Description:

Objectives:

Study the pathology of schistosome infections with special reference to portal collateral circulation, including esophageal varices. Study the passage of ammonia from the blood to the brain. Study the consequences of schistosome infections in man.

Methods Employed:

Study changes in the portal circulation of mice and monkeys at intervals following infection, by dissection, by measurement of portal pressure, by X-ray following injection of opaque material into the portal system, by injection of colored latex into the system, and by the study of liver tissue sections. Production of toxic cirrhosis with CCl_4 and other chemicals in order to compare it with that produced by schistosomiasis. Simultaneously determine blood and brain ammonia levels.

Major Findings:

A syndrome that closely resembles hepato-splenic schistosomiasis in humans was reproduced in mice. It was determined that the pathogenesis of the syndrome was related to the penetration of the portal canals by large numbers of schistosome eggs causing obstruction of portal blood flow. No specific alteration in parenchymal liver cell function was detected.

Significance of the Program to the Institute:

Schistosome infections in mice and other animals provide a means to study cirrhosis, collateral circulation, and ammonia metabolism.

Proposed Course of the Project:

Continue study on the pathology of schistosome infections with special reference to the cause and characteristics of the severe anemia that develops in mice infected with Schistosoma mansoni. Attempts will be made to reproduce the hepato-splenic syndrome in dogs.

Part B included - Yes

Serial No. NIAID-121-I

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Warren, K. S and DeWitt, W. B.: The production of portal hypertension and esophageal varices in the mouse. Proc. Soc. Exper. Biol. and Med., 98: 99-101, 1958.

Warren, K. S and DeWitt, W. B.: Esophageal varices in mice infected with Schistosoma mansoni. Proc. 6th Internat. Congr. Trop. Med. and Malaria. (in Press).

Warren, K. S and Nathan, D. G.: The passage of ammonia across the blood-brain-barrier and its relation to blood pH. J. Clin. Investig. (in press).

Stabenau, J. R., Warren, K. S and Rall, D. P. The role of pH gradient in the distribution of ammonia between blood and cerebrospinal fluid, brain, and muscle. J. Clin. Investig. (in press).

Nathan, D. G. and Warren, K. S A colorimetric method for the measurement of the brain ammonia of the mouse: The effect of glutamine on the total measurable ammonia. Arch. Biochem. and Biophys. (in press).

Honors and Awards relating to this project: None

Serial No. NIAID-122-A
1. Tropical Diseases
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biochemical pharmacology of antimalarial drugs and related compounds

Principle Investigator: Leo E. Gaudette

Other Investigators: None

Cooperating Units: None

Man years (Calendar year 1958)

Total:	1
Professional:	2/3
Other:	1/3

Project Description:

Objectives:

To define the metabolic pathways and enzyme systems active on antimalarial agents; to elucidate the physical and chemical properties influencing the physiological disposition of these drugs; to determine the usefulness of salt derivatives and salt combinations as effective means of mass therapy; and to determine the toxicology and pharmacology of various drugs found effective as antimalarials in a general screening program.

Methods Employed:

P. berghei in the mouse and P. gallinaceum in the chick are the malaria strains used primarily. The drugs most intensively studied are pyrimethamine and various quinolines. In vivo and in vitro studies are accomplished in mouse, rat, and rabbit. Specific chemical methods are developed for the determination of parent compound and/ or metabolites in urine, feces, plasma and tissues.

1. Physiological disposition of drugs labelled with radioisotopes is determined in animals after oral and parenteral administration. Tissue distribution, plasma levels, excretion rates and metabolic products are determined by specific chemical methods and paper chromatography.

2. The metabolism of antimalarials is determined in tissue preparations. Enzyme systems are characterized by classical and specialized biochemical techniques.

3. The chemical properties of varied salt derivatives and salt combinations with antimalarial drugs are determined. The properties of various binders are defined for extent and reversibility of binding, toxicology and pharmacology.

4. Numerous compounds structurally related to known antimalarial agents are screened for antimalarial activity. Inhibitors of drug metabolism are studied as possible potentiators of antimalarial activity.

Major Findings:

Tritiated Daraprim was administered parenterally to the rabbit. The compound was subsequently excreted at a very slow rate. In addition to the parent compound, two prominent metabolites were found excreted in the urine. Tritiated Daraprim was given orally to the mouse. About 75 per cent was excreted unchanged in the feces; the remainder was distributed in plasma, liver, kidney, lung and urine. Oral administration of the free base was found unsatisfactory, giving rise to unpredictable plasma levels and side-reactions, undoubtedly a reflection of individual variations in absorption.

Various salts of Daraprim having a wide range of pK_a's were investigated as potential compounds with which to obtain more predictable, constant, and reproducible plasma levels. Preliminary results suggest that derivatives with a pK_a of about 4.5 - 5.0 may be desirable for this purpose, as in the case of Daraprim-acetate and Daraprim-succinate.

The binder, Magnesol, presently used in chloroquinized-salt mixtures for mass therapy was studied for retention of the antimalarial drug in such mixtures. It has been found that in moist areas the binder does not prevent the antimalarial from being leached out of the mixture; suggestions were made to increase its concentration. It was found that under such conditions, an irreversible binding of about 40 per cent of the drug occurs, posing economic problems, as well as problems regarding its therapeutic value.

Significance to the Program of the Institute:

The project is associated with a larger one aimed at the eradication of malaria. The purpose of the program is to find a drug or drug combination with prolonged action, maximum therapeutic value, and minimal toxicity, plus a simple "foolproof" method of administration.

Proposed Course of the Project:

1. Metabolic studies of antimalarial agents will continue in vivo and in vitro. Urinary metabolites will be investigated for identification, antimalarial activity, and toxicity. The enzyme systems of detoxication of antimalarials will be investigated further, giving consideration to their possible inhibition and the potentiation of antimalarial drugs by such inhibitors.

2. Greater emphasis will be placed on the studies of salt combinations and derivatives. Binders will be sought as effective additives, but with reversible binding properties under physiological conditions.

3. Toxicology and pharmacology of various antimalarials and drug combinations will be undertaken to better understand the drug and drug action.

4. Screening program will continue of various compounds structurally related to presently known antimalarials, but with reduced toxocity.

Part B included - No.

Serial No. NIAID - 122 - B
1. Tropical Diseases
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Mode of action of therapeutic agents

Principal Investigator: Karl Schellenberg

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To find out the mechanism of action of antimalarial drugs; to determine the biochemical properties of plasmodia which make it an obligate intracellular parasite; and the influence of such requirements on effective drug therapy.

Methods Employed:

1. The metabolism of the parasites P. gallinaceum and P. berghei and/or host is being explored at all levels from the intact organism to model enzyme systems, noting the effects of the representative drugs quinine, atabrine, chloroquine, primaquine, pyrimethamine, and proguanil.

(a) A study of the respiration of parasites under varied near-physiological conditions is made. The direct effect of representative antimalarial drugs at various concentrations is determined.

(b) Model systems for DPNH oxidation are established in which the antimalarials and their free-radical forms are tested as oxidants to DPNH, and consequently possible antagonists to flavo-enzymes.

(c) The DNA and RNA content of normal and infected chicken and mouse blood are determined as a baseline for future investigation

of the inhibition of DNA synthesis by antimalarial drugs.

2. The maintenance and cultivation of P. gallinaceum in vitro is being undertaken and the determination of a chemically defined medium is sought.

Major Findings:

1. The presence of the intact red cell enclosing the parasite has been found necessary for the inhibition of respiration of parasites by quinine. This is in agreement with the findings of quinine inhibition of DNA synthesis by the parasite.

Hemolysis by specific serum, saponin, or hypotonicity progressively abolished the quinine effect in proportion to the degree of hemolysis. Variation in parameters of parasite environment such as ionic composition, pH, presence of lipids and proteins did not materially change the action of quinine.

2. Antimalarials with quinoline and acridine nuclei, as well as other compounds capable of forming quinones, but inactive against malaria, formed active oxidants for DPNH. Proguanil and pyrimethamine did not.

Significance to the Program of the Institute:

Biological and biochemical information related to the mechanism of action of antimalarial drugs will afford basic knowledge in an area presently little understood in the chemotherapy of parasitic disease. This information will allow the synthesis of more effective and specific drugs.

In addition, such information will be of inestimable value in other areas of research in which the use of antimalarials has been found beneficial--as heart disease, arthritis, and lupus in the case of the quinoline group. Where folic acid antagonists are of therapeutic value, as in the case of leukemia, pyrimethamine may be of some value.

Proposed Course of the Project:

1. Metabolic studies will continue. The inhibition of parasite respiration by antimalarials will be employed as an index for further detailed metabolic investigations at the enzyme level.

2. Using radiophosphorous, a study of DNA and RNA synthesis in the parasite, along with the effect of antimalarials thereon will be undertaken.

3. Exhaustive attempts will be made to cultivate the parasite in vitro in a chemically defined medium free of host influence, in order that the more subtle influences of drugs and their metabolites may be determined.

Part B included - No

Serial No. NIAID-122-C
1. Tropical Diseases
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar year 1958

Part A.

Project Title: Mode of action of therapeutic agents.

Principle Investigator: Joseph Greenberg

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1958):

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

To find out how chemical agents kill animal parasites; to determine the way these organisms become resistant to such chemicals, how this resistance is transmitted from generation to generation, and the effect of such resistance on other biological properties of the organism.

Methods Employed:

(a) Plasmodium gallinaceum in the chick and Plasmodium berghei in the mouse and rat are used primarily. The drugs most intensively studied are diaminopyrimidines and various quinolines. The antimalarial activity of the drugs as complexes and in the presence of various potentiators is determined. Specific metabolic pathways are studied to determine the presence of any inhibition by these drugs; and a comparison made between normal and resistant parasites as to the presence or absence of such pathways.

(b) Strains are developed which are resistant to chemical agents. Strains are also developed which have other distinct, stable biological properties, such as sexuality, lethality,

tissue preference, and host specificity. These various strains are hybridized by mass mating in mosquitoes and rearrangements in the strain characteristics are observed.

(c) Plasmodia are maintained in tissue culture and the effects of therapeutic agents are studied under the controlled conditions of the test tube.

Major Findings:

Preliminary results suggest that the antimalarial activity of such compounds as chloroquine may be prolonged when administered in conjunction with an inhibitor to the metabolism of the drug, which is otherwise innocuous.

Studies with a sulfanilic acid complex of chloroquine indicated a fourfold decrease in toxicity of the antimalarial drug; on the other hand a fourfold increase in concentration had to be administered to obtain the same therapeutic level. However, such a compound may have application in cases of prolonged therapy.

Significance to the Program of the Institute:

(a) The project is intimately associated with a larger project aimed at eradication of malaria. The purpose of the project is to conduct basic studies on problems of drug metabolism and drug resistance which may be significant factors in limiting the effectiveness of the larger program.

(b) A deficiency exists in our information about the mechanism of drug resistance. This project is designed to answer some of the questions in this field.

(c) The mode of action of therapeutic agents is little understood. The knowledge derived from studying tissue cultured parasites and effect of drug action on them is designed to fill this deficiency

Proposed Course of the Project:

The project as outlined has been discontinued as a result of the resignation of the major investigator. However, various phases have been undertaken and described in the annual reports of other investigators of this laboratory.

Part B included - Yes.

Serial No. NIAID-122-C

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Greenberg, J. and Kendrick, L.: Parasitemia and survival in mice infected with Plasmodium berghei. Hybrids between Swiss (high parasitemia) and Str (low parasitemia) mice. J. Parasit., 44: 492(1958).

Greenberg, J. and Kendrick, L.; Resistance to malaria in hybrids between Swiss and certain other strains of mice. J. Parasit., (In Press).

Serial No. NIAID - 122-B
1. Tropical Diseases
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Experimental chemotherapy of helminthic diseases

Principal Investigator: George W. Luttermoser

Other Investigators: None

Cooperating Unit: Dr. B. Prescott, LID, NIAID-66

Man years (calendar year 1958):

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

To discover new and more effective schistosomacida. compounds; how best to administer them; and how they selectively kill worms.

Methods Employed:

Mice are infected with cercariae of Schistosoma mansoni and treatment begun (a) immediately (prophylaxis), or (b) after 35 days (curative). The mice are examined 50 days or more after infection for the presence of dead worms. If these are found, the liver and portal systems are perfused and live worms counted. Live mature worms are removed from treated and untreated animals, and are observed in maintenance cultures for activity and survival. Drugs are added to the cultures. Mouse pinworms are also studied under similar conditions.

Major Findings:

Of the ninety-four compounds tested during the year, two were selected for extensive trials. One was the phenylpiperazine (Hoechst 688) previously found to have strong curative activity against Schistosoma mansoni infection in mice. This drug almost completely cured a monkey with the same infection when given at a relatively high dose. However, it did not reduce the S. japonicum infection in a dog which had received a lower dose. Attempts to enhance or detoxify the drug

were unsuccessful. This compound also prevents mice from acquiring the S. mansoni infection if given orally on each of three days subsequent to exposure. Prophylactic trials in larger animals are in progress. Sera and urine from mice one-half and one hour after oral administration of the drug contain a substance lethal for normal schistosomes in cultures.

The second of the two compounds was an analogue of Miracil D. Numerous trials in mice indicated its lower toxicity but also lower activity than the parent compound with the result that there probably was no improvement in the therapeutic index. The fact that Miracil D can be given in this form for a longer period of time has possible clinical application.

Culture conditions for maintaining the schistosomes and pinworms active for 48 hours have been defined. These conditions have been found satisfactory for observing in vitro the effects of drugs active in vivo. The cultures have also been used successfully as a bioassay for detecting active drugs in blood and urine of treated animals.

Significance to the Program of the Institute:

Current treatment for schistosomiasis is inadequate. Programs aimed at eradication or reduction of snails leave a large human population already infected as potential agents of transmission should the snail control program fail or abate. Further, little is known about the mode of action of currently available drugs, their absorption, excretion, tissue distribution, metabolism, and in vitro activity.

Proposed Course of the Project:

Screening will continue in an effort to find more promising compounds. A greater effort will be made to elucidate how presently known compounds kill in vivo and in vitro. An understanding of the mode of action may give promising leads as to possible new drugs. Emphasis will be placed on the anti-cholinergic activity of various drugs.

Part B included - Yes

Serial No. NIAID-122-D

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Luttermoser, G. W.: Studies on chemotherapy of experimental schistosomiasis V. Enhancement of the schistosomacidal activity of tartar emetic and stibophen by glycerin. J. Parasit. (in press).

Honors and Awards relating to this project: None

Serial No. NIAID-122-E
1. Tropical Diseases
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Experimental chemotherapy of malaria

Principle Investigator: Micah Krichevsky

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958)

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To determine metabolic processes of the malaria parasite; to study the effect of therapeutic agents on these metabolic pathways; to find the differences in the metabolism of normal and resistant parasites, and how this resistance is transmitted from generation to generation.

Methods Employed:

Plasmodium gallinaceum in the chick is used primarily. Specific metabolic pathways of the parasite are studied and the activity thereon of various antimalarial drugs is determined. Comparisons are made between normal and resistant parasites as to the presence or absence of such pathways, and their susceptibility to the therapeutic agent. The studies are performed with whole cells and cell-free extracts, and quantitative aspects are evaluated by means of radioisotopes.

Strains are developed which are resistant to chemical agents. The strains are hybridized by mass mating in mosquitoes and rearrangements in the strain characteristics are observed at the enzymatic level.

Methods are developed for the cultivation of sporozoites and the effects of drugs when studied in vitro.

Major Findings:

The major investigator has only recently joined the staff of the laboratory and the research program has just begun.

Significance of the Program to the Institute:

1. The project is associated with one aimed at the eradication of malaria. The purpose of the project is to conduct basic studies on problems of drug resistance which are of inestimable value in the area of developmental malaria chemotherapy.

2. A lack of understanding of the mechanism of drug resistance is apparent. The project is designed to gain some insight into this area, and how this resistance is transmitted from generation to generation.

Part B included - No.

Serial No. NIAID-123-A
1. Tropical Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Fundamental physiological and biochemical studies on parasites, intermediate hosts, and parasitized animals

Principal Investigator: T. von Brand

Other Investigators: E. J. Tobie, P. A. McMahon

Cooperating Units: 1. Section on Steroids, Laboratory of Chemistry, NIAMD

2. Laboratory of Histology and Pathology, NIDR

Man Years (calendar year 1958):

Total:	4 3/4
Professional:	2 3/4
Other:	2

Project Description:

Objectives:

The objective is to provide knowledge on the chemical composition, the metabolism, and the biology of parasites.

Methods employed:

Trypanosomes are maintained for certain periods of time in vitro and their metabolic products, as well as those of Cysticercus fasciolaris (obtained from the Section on Helminthic Diseases), are studied by manometric and chemical techniques. Strains of trypanosomes are mixed and their infectivity and behavior in experimental animals observed. Vertebrate and invertebrate hosts of T. rangeli are infected by syringe passage and various methods of transmission studied. T. taeniaeformis and C. fasciolaris are processed for isolation of lipids or calcareous corpuscles. The latter are prepared for x-ray and electron diffraction studies in collaboration with the Laboratory of Histology and Pathology, NIDR. Lipids are chromatographically

separated and the components of the phospholipid fraction are identified by chromatography and electrophoresis of their hydrolytic products. The unsaponifiable material from T. taeniaeformis and Moniezia sp. (mainly M. expansa) are isolated for study in collaboration with the Section on Steroids, NIAMD.

Major Findings:

Mixing of an arsenic-sensitive and an arsenic-resistant strain of Trypanosoma leads to slow decline of the resistant strain, apparently due to minor differences in their multiplication rates. The relative carbohydrate content of Cysticercus fasciolaris from rats exceeds that from mice, with glycogen accounting for only 50% of the total carbohydrate. C. fasciolaris produces detectable quantities of succinic, lactic, pyruvic acids, glycerol and ethanol as end products of its carbohydrate metabolism. Some of the hydrolytic products of the phospholipid fraction of T. taeniaeformis have been tentatively identified as serine, ethanolamine, choline and oleic acid.

Significance to the Program of the Institute:

The studies on metabolism and chemical composition of parasites are essential for an understanding of the pathogenesis of parasitic diseases. Biological studies frequently are a prerequisite for subsequent specialized studies which provide a basis for a rational approach to chemotherapy.

Proposed Course of the Project:

Investigation of the metabolism and chemical composition of trypanosomes and larval tapeworms will be continued. The biology and life cycle of Trypanosoma rangeli will be studied. Work on chemical composition of the lipids of parasites will be pursued with emphasis on phospholipids. Study of the unsaponifiable material will continue with collaboration of the Section on Steroids, NIAMD. Crystalline structure of calcareous corpuscles will be investigated further with the cooperation of the Laboratory of Histology and Pathology, NIDR.

Part B included - Yes

Serial No. NIAID-123-4

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

McMahon, Patricia; von Brand, Theodor and Nolan, M. O.: Observations on the polysaccharides of aquatic snails. J. Cellular and Comp. Physiol. 50: 219-240, 1958.

Tobie, Eleanor Johnson: The cultivation of Trypanosoma congolense. J. Parasitol. 44: 241-242, 1958.

Perlowagora-Szumlewicz, Alina, and von Brand, Theodor: Observations on the oxygen consumption of young Australorbis glabratus. J. Washington Acad. Science 48: 38-43, 1958.

Tobie, Eleanor Johnson and Higman, Benjamin: Failure to demonstrate cerebral lesions in rabbits infected with Trypanosoma gambiense. J. Parasitol. 44: 273, 1958.

von Brand, Theodor, and Tobie, Eleanor Johnson: Observations on the metabolism of the culture form of Trypanosoma congolense. J. Parasitol. (In press).

von Brand, Theodor; McMahon, Patricia; Tobie, Eleanor Johnson; Thompson, Malcolm J., and Mosettig, Erich: Observations on the composition of the culture form of Trypanosoma cruzi. Exptl. Parasitol. (In press).

Honors and Awards relating to this project:

Dr. Theodor von Brand was appointed to the General and Physical Biology Panel of the National Institutes of Health Central Qualifications Board.

Dr. Theodor von Brand was invited to present a series of lectures in West Germany as a guest of the Bavarian Ministry of Education.

Serial No. NIAID-123-B
1. Tropical Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pathological physiology

Principal Investigator: T. von Brand

Other Investigators: E. C. Weinbach, T. I. Mercado

Cooperating Units: Laboratory of Physical Biology, NIAMD.

Man Years (Calendar year 1958):

Total:	2 3/4
Professional:	1 1/2
Other:	1 1/4

Project Description:

Objectives:

The objective is to elucidate the pathogenic action of parasites on their hosts and to study the mechanisms of action of drugs.

Methods Employed:

Normal rats and rats infected with blood parasites (Bartonella muris, Trypanosoma equinum, T. congolense) are starved or fed with fructose, untreated or treated with meticortetone and the liver glycogen and lipids studied histochemically. The effect of pentachlorophenol on the ATPase and contractile properties of psoas muscle models is determined by biochemical and physiological techniques.

Major Findings:

Administration of steroid partially relieves the liver glycogen depletion observed in highly anemic rats infected with Bartonella muris. Glycogen synthesis increases when the animals receive fructose with the steroid, but the increase is less than

previously observed with malaria. The patchy glycogen deposition around the portal tracts, commonly observed in malaria, did not occur in B. muris or in T. equinum infections. In the latter infection where little anemia occurs, glycogen depletion is greater than in the case of B. muris but the administration of fructose and steroid increases glycogen synthesis. Centrilobular lipid infiltration was observed in both B. muris and T. congolense infections. Pentachlorophenol exerts a dual action upon myosin ATPase similar to its effect upon mitochondrial ATPase; low concentrations of the phenol enhance phosphatase activity and high concentrations inhibit. The halophenol when used alone, induces contraction of psoas muscle models, but it powerfully inhibits the contraction induced by ATP.

Significance to the Program of the Institute:

The studies of disturbed liver function in infected animals contribute to an understanding of the pathogenesis of parasitic diseases, and in addition, provide a rational basis for possible therapeutic measures. The biochemical studies of pentachlorophenol's effect on muscle metabolism contributes both to basic problems of muscular contraction and to further understanding of the toxic action of this important compound.

Proposed Course of the Project:

The histochemical studies will be extended to include enzymatic techniques. The preliminary experiments are now under way. The heart muscle of rats infected with T. cruzi will be studied by histochemical and electrocardiographic techniques. Investigation of the mechanism of pentachlorophenol's interaction with catalytic proteins will be continued.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Von Brand, Theodor and Mercado, Teresa: Quantitative and histochemical studies on liver lipids of rats infected with Plasmodium berghei. Am. J. Hyg. 67, 311-320, 1958.

Weinbach, Eugene C. and Bowen, William J. The effect of pentachlorophenol on the adenosinetriphosphatase and contractile properties of psoas-muscle preparations. Biochem. and Biophys. Acta 30: 476-484, 1958.

Honors and Awards relating to this project: None

Serial No. NIAID-1.3- C
1. Tropical Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biochemical mechanisms of energy metabolism in normal, aged, and parasitized animals.

Principal Investigator: E. C. Weinbach

Other Investigators: None

Cooperating Units: Section on Aging, Laboratory of Psychology, NIMH-M-P-A-13

Man Years (calendar year, 1958):

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

The objective is to study the biochemical changes in energy metabolism associated with biological aging and parasitism.

Methods Employed:

Mammalian tissues are fractionated by mechanical disruption of the cells in suitable media and the subcellular elements (mitochondria, nuclei, soluble fraction) isolated by differential centrifugation. Enzymatic exergonic and related reactions are studied with preparations obtained from normal young animals. Coenzyme levels are determined by appropriate enzymatic and spectrophotometric techniques. Radioisotope procedures are used to investigate mechanisms of oxidative phosphorylation such as the P^{32} ATP exchange reaction. In collaboration with the Section on Aging, NIMH, similar studies are conducted with tissues from senescent rats.

Major Findings:

Lowered contents of pyridine and adenine nucleotides were found in brain and liver mitochondria of the aged rat. This may be related to the impaired oxidative phosphorylation reported previously. Measurements of the P^{32} ATP exchange revealed no difference in activity between young and old animals, thus precluding a transphosphorylation sequence as the site of the observed decline in oxidative phosphorylation. Studies of the stability of oxidative phosphorylation in isolated mitochondria disclosed that under certain conditions of pretreatment such as low temperature storage, rat liver mitochondria retain their capacity for coupled phosphorylation but no longer catalyze the P^{32} ATP exchange. This lack of parallelism between the two processes is of importance in mechanism studies of oxidative phosphorylation.

Significance to the Program of the Institute:

This investigation, in addition to its obvious contribution to the biochemistry of senescence, serves as an important prototype for future studies of energy changes associated with parasitism.

Proposed Course of the Project:

The biochemical studies of senescence will be continued, but at a lower rate during the coming year. Emphasis will be shifted toward initiating an investigation of biochemical energetics in parasitized animals.

Part B included - Yes

Annual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinbach, Eugene C. Biochemical changes in mitochondria associated with age. Gatlinburg Conference on Basic Biological Research in Aging. In press.

Weinbach, Eugene C. and Garbus, J. Oxidative Phosphorylation in mitochondria from aged rats. J. Biol. Chem. In press.

Honors and Awards relating to this project:

Dr. E. C. Weinbach was appointed to the Biochemistry and Nutrition Fellowship Review Panel of the National Institutes of Health Central Qualifications Board.

Serial No. NIAID-124-A
1. Tropical Diseases
2. Germ-free Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Virulence studies in trichomoniasis

Principal Investigator: Walter L. Newton

Other Investigators: Anna M. DeLeva

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2 1/6
Professional:	2/3
Other:	1 1/2

Project Description:

Objectives:

To determine, through the use of germ-free animals, whether virulence difference exists among strains of Trichomonas vaginalis.

Methods Employed:

T. vaginalis derived from human infections of varying severity are maintained in axenic cultures. These organisms are inoculated into young germ-free guinea pigs at various sites. Animals are followed for evidence of infection and tissues are examined at necropsy.

Major Findings:

Thus far, it has not been possible to establish the parasite in the genital tract of guinea pigs. However, the preliminary results obtained previously with subcutaneous inoculations were confirmed and extended.

A marked difference was obtained in the pathology produced subcutaneously in germ-free pigs by two strains of T. vaginalis derived originally from cases of differing severity. With the "mild" strain there was little evidence of the inoculation at 2 weeks, whereas the more virulent strain produced large gas-filled ulcerous lesions containing numerous organisms. Spectrometric analysis showed that considerable quantities of hydrogen were produced by this organism.

These marked differences were not noted in conventional guineas of the same age on the same diet. Little or no evidence of the inoculation remained, even with the more virulent strain.

Significance to the Program of the Institute:

The demonstration that there can be considerable variation in the virulence of the parasite per se can aid in the further understanding of the pathogenesis of Trichomoniasis vaginalis. Of considerable interest, also, from the immunologic standpoint was the finding that, although the deep subcutaneous area of the conventional animal should presumably have been as "sterile" as that in the germ-free animal, only in the latter did the parasites prosper. These studies point up the potential value of the germ-free animal in bringing to light subtle differences among strains of organisms.

Proposed Course of the Project:

A few more attempts, perhaps with the use of hormones, will be made to establish this parasite in the genital tract. If unsuccessful this project will be discontinued. Scientists from another institute have expressed an interest in studying certain physiologic aspects of the gas bubbles produced by this parasite in the germ-free animal. If sufficient animals become available, an experiment or two along this line may be undertaken.

Part B included - Yes

PHS -NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Wright, Willard H., Phillips, B. P., and Newton, W. L.: Germ-free animal research at the National Institutes of Health. Proc. VIIth Internat. Cong. for Microbiol. (In press).

Honors and awards relating to this project: None

Serial No. NIAID-124-B
1. Tropical Diseases
2. Germfree Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calenday Year 1958

art A:

Project Title: The pathegenesis of amoebiasis

Principal Investigator: Bruce P. Phillips

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1
Other:	1 (includes ½ for night and weekend personnel)

Project Description:

Objectives:

To study amoeba-bacteria relations in the etiology, pathogenesis and pathology of intestinal amoebiasis

Methods Employed:

Germfree animals are inoculated with trophozoites from cultures of Entamoeba histolytica and various known species of intestinal bacteria or bacterial products. The intestines are examined grossly and microscopically for lesions. Although amoebae were shown to live in a localized walled-off lesion in the gut wall for months. Acute ulcerative amoebiasis was obtained only when live bacteria were present. Evidence has been obtained which suggests that the species of bacteria may help determine the kind of pathology produced.

Significance to the Program of the Institute:

Studies on amoebiasis in germfree animals can provide information on the invasiveness of the amoeba per se, and the role of bacteria

in the maintenance of the parasite and the pathogenesis of the disease. This information will aid in the solution of such problems as the nature of the observed variation in pathogenicity, the question of whether this parasite always invades tissue, and the nature of the effect that various diets and chemotherapeutic agents have upon the course of infection.

Proposed Course of the Project:

This project was interrupted for several months as the result of its transfer from the Lobund Institute to the NIH. The effect of different species of intestinal bacteria, e.g., whether they merely maintain the parasite or contribute to the formation of ulcers and abscesses, will be studied further in immunized and non-immunized monocontaminated hosts. Also, attempts will be made to isolate factors which will aid in the establishment of E. histolytica in the lumen of the germ-free animal.

Part B included - Yes

Serial No. NIAID-124-B

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Phillips, B. P., Wolfe, P. A., and Bartgis, I. L. Studies on the ameba-bacteria relationship in amoebiasis II. Some concepts on the etiology of the disease. Am. J. Trop. Med. Hyg. 7: 392-399, 1958.

Phillips, B. P., and Wolfe, P. A. The use of germfree guinea pigs in studies on the ameba-bacteria relationship in amebiasis. Ann. N. Y. Acad. Sci. (In Press).

Phillips, B. P., Wolfe, P. A., and Gordon, H. A. Studies on rearing the guinea pig germfree. Ann. N. Y. Acad. Sci. (In Press).

Honors and Awards relating to this project: None

Serial No. NIAID-124-C
1. Tropical Diseases
2. Germ-free Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Studies on helminthic infections in germ-free hosts

Principal Investigator: Walter L. Newton

Other Investigators: A. M. DeLeva

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2 1/6
Professional:	2/3
Other:	1 1/2 (includes 1/2 for night and weekend personnel)

Project Description:

Objectives:

To ascertain what effect the bacteria and other contaminants in the conventional animal have upon the course of infection of, and host response to, parasitic helminths.

Methods Employed:

Germ-free animals are fed or inoculated with sterilized eggs, or axenically-reared infective larvae, of various parasitic helminths. The development of these parasites is followed by established procedures, and lungs, intestine, and other appropriate tissues are examined histologically for the study of host response.

Major Findings:

The incidental observation, made previously, that the mouse tapeworm, Hymenolepis nana, could develop to maturity in the germ-free guinea pig (an abnormal host) was confirmed. While worm yields were low and variable, they ran as high as 10%. Apparently mature specimens as large as those reported for the mouse were recovered. In conventional guinea pigs, fewer and only immature forms were recovered.

Ascaris lumbricoides developed no further in the germ-free than in conventional guinea pigs. There was gross evidence of lung damage caused by the migrating larvae in both instances, although the comparative histopathology is not yet available.

Significance to the Program of the Institute:

These studies can lead to an understanding of the influences bacteria exert upon the establishment and course of helminth infections. The fact that bacteria, *per se*, are apparently not required for full development of some of these parasites opens the way for other studies in the areas of nutrition and parasitism, chemotherapy, *in vitro* cultivation, and physiological studies. Of special significance is the finding that some parasites were more "successful" in germ-free abnormal hosts than in the contaminated abnormal one. These findings could provide an approach to the study of the concepts of natural immunity and host specificity, in general.

Proposed Course of the Project:

The study of helminth infections in germ-free animals will be continued, and both normal and abnormal hosts will be employed. Comparative tissue and blood studies of germ-free and conventional hosts will be continued in an effort to ascertain differences which could be correlated with infection results.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Newton, Walter L., Weinstein, Paul P., and Jones, Myrna F.: A comparison of the development of some rat and mouse helminths in germfree and conventional guinea pigs. Ann. New York Acad. Sci. (In press).

Honors and Awards relating to this project: None

Serial No. NIAID-124-D
1. Tropical Diseases
2. Germ-free Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Basic studies on germ-free animals

Principal Investigator: Walter L. Newton

Other Investigators: Richard E. Horton

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1 1/3
Professional:	1/3
Other:	1 (including 1/2 for night and weekend personnel)

Project Description:

Objectives:

To obtain basic data descriptive of the germ-free animal, and to determine characteristics in which the latter differs from the conventional animal.

Methods Employed:

Germ-free animals are studied from such standpoints as serum protein analysis, complement activity, blood chemistry, intestinal histology, and morphology. Standard laboratory procedures, e.g., paper electrophoresis techniques, are followed.

Major Findings:

Studies involving a total of 81 young germ-free and conventional guinea pigs, on the same diet, showed essentially no differences in the levels of the various serum protein fractions and in complement activity. There was a significant reduction in the gamma globulin fraction and a proportionate increase in the alpha fraction between the first and second week of life in both groups. All values were below those of older, mature conventional guinea pigs.

Increases in dietary protein raised portal blood ammonia levels in germ-free guinea pigs, but not nearly to the extent raised in conventional animals with intestinal bacteria. Peripheral blood levels

were not appreciably affected by dietary protein changes. Of interest was the fact that germ-free animals on a protein-free diet had portal ammonia levels which averaged slightly above those of the peripheral blood.

Conventional guinea pigs delivered by Caesarean section and not provided the opportunity to suckle tended to develop enlargement of the cecum in early life, just as do the germ-free guinea pigs.

Significance to the Program of the Institute:

These studies provide baseline data for experiments involving germ-free animals. They can give some insight, also, into the effects which constant association with living micro-organisms has upon the physiology, histology, and immunologic processes of an animal.

Proposed Course of the Project:

It is expected that fundamental studies of a broad nature will be continuously undertaken as the opportunity is provided. The serum protein picture of older mature germ-free guinea pigs will be studied as the latter become available. The significance of the enlarged cecum will be investigated.

Part B included - No

Serial No. NIAID-124-E
1. Tropical Diseases
2. Germ-free Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

t A:

Project Title: Development and improvement of germ-free animal experimentation

Principal Investigator: R. E. Horton

Other Investigators: A. M. DeLeva, W. L. Newton

Cooperating Units: Sanitary Engineering Branch, SEB-8

Man Years (calendar year 1958):

Total:	1 5/6
Professional:	1/3
Other:	1 1/2 (includes ½ night and weekend personnel)

Project Description:

Objectives:

To improve the quality and availability of germ-free animals and broaden their application in the research program of the Institute.

Methods Employed:

Improved diets and means of sterilization (eg, irradiation) are tried. Attempts are made to rear or colonize new species. Procedures are developed to check the status of the germ-free animals with regard to swab agents as viruses, and to use the latter in experiments.

Major Findings:

A colony of germ-free white swiss mice has been established from a few sent from the Lobund Institute.

Tests with high concentrations of bacteriophage, (as a virus type) introduced in the incoming air stream as an aerosol or fine powder indicated that the filtering device on the Reyniers germ-free units was very effective. For all practical purposes, contamination of the germ-free animals with virus from incoming air seems unlikely, under normal oper-

animals with virus from incoming air seems unlikely, under normal operating conditions. Tests are underway to determine whether viruses can be detected in the germ-free mouse colony.

The dose of irradiation necessary to sterilize stock diets for germ-free guinea pigs has been determined. Conventional guinea pigs maintained on irradiated diets from 2 days of age have shown optimal growth and development. Thus far, germ-free guinea pigs have been reared for 6 weeks on such a diet.

Significance to the Program of the Institute:

If germ-free animals are to be useful tools in various studies within the program of the Institute, it is essential that there be a variety and that they be vigorous and as standard as possible. While we have a diet on which germ-free guinea pigs can be raised, the results are variable and the animals usually do not compare well with conventionals. This may be in part due to diet, the use of steam sterilization, or to the lack of suckling. In studies with viral agents, it would be of interest to know just how "germ-free" the animals are.

Proposed Course of the Project:

Efforts will continue toward improving the guinea pig, particularly during early life. The mouse colony will be surveyed for viruses of the more common types and for which cell lines and test procedures are available. If more equipment becomes available and if interest in these animals warrants, efforts to initiate a small rat colony and to have chickens may be undertaken.

Part B included - No

Serial No. NIAID-124 F
1. Tropical Diseases
2. Germ-free Animal Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Miscellaneous short-term exploratory experiments with germ-free animals

Principal Investigator: W. L. Newton

Other Investigators: None

Cooperating Units: Laboratory of Microbiology - NIDR
USPHS Hospital, Carville, Louisiana
Laboratory of Infectious Diseases - NIAID - 55

Man Years (calendar year 1958)

Total:	1 5/6
Professional:	1/3
Other:	1 1/2 (includes 1/2 for night and weekend personnel)

Project Description:

Objectives:

In cooperation with scientists from other units, to conduct exploratory experiments to determine such things as whether a given organism behaves differently, an enzymatic activity is present or absent, or an immune phenomenon follows a different course, in germ-free animals, as compared with conventional ones.

Methods Employed:

Germ-free animals are inoculated with specific organisms and the presence and course of infection followed by appropriate test procedures and tissues, e.g., from germ-free intestine, are introduced into a specific enzyme test-system.

Major Findings:

Plasmodium berghei from the rat, Trypanosoma lewisi from culture, and various oral spirochaetes from culture failed to become established when injected into either germ-free or conventional guinea pigs. Mycobacteria from a human leprous lesion were injected intracutaneously

into germ-free guinea pigs with the hope that better infections than what usually occurs in laboratory animals might be obtained. Specimens up to 6 weeks post-inoculation have shown only small granulomas containing acid-fast bacilli, results of which may be interpreted as essentially negative. Of interest, however, is the fact that human material from such a lesion could be obtained and introduced into the germ-free system without contamination.

The intestine from young germ-free guinea pigs was shown to have strong beta-galactosidase activity, just as in the case of young conventionals. The question had been raised as to whether bacteria might be the source in the latter.

Significance to the Program of the Institute:

It is essential that short-time exploratory experiments of this kind be done, from time to time. They can serve as a source of leads to new approaches in the various projects underway within the Institute and serve as means of evaluating the contaminative factor in projects involving animal studies.

Proposed Course of the Project:

It is anticipated that these experiments will continue, within the limits of the available germ-free equipment and the needs of the other, more intensive projects.

Part B included - Yes

Serial No. NIAID-124-F

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Fitzgerald, R. J., Hampp, E. G., and Newton, W. L.: Infectivity of oral spirochaetes in cortisone--treated and germfree guinea pigs. J. Dental Res. 37: 11, 1958.

Honors and Awards relating to this project: None

Serial No. NIAID-125-A
1. Tropical Diseases
2. Middle America Research Unit
3. Ancon, C. Z.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Studies of viral infections, especially due to arthropod-borne agents, in the tropics of the Western Hemisphere

Principal Investigator: Alexis Shelokov

Other Investigators: John E. Craighead, John E. Vogel, Pauline H. Peralta

Cooperating Units: Gorgas Memorial Laboratory
Laboratorio de Diagnostico e Investigacion Veterinaria
U. S. Malaria Control and Survey Branch, Corozal
Epidemiology Section, Dept. of Health, R. de P.
Gorgas and Coco Solo Hospitals
Laboratorio Nacional
Santo Tomas Hospital
Health Director
Chief Surgeons for the Army, Navy, and Air Force of the Caribbean Command
Ministry of Health

Man Years (calendar year 1958):

Total:	4½
Professional:	1½
Other:	3

Project Description:

Objectives:

To evaluate the significance of viral agents found among the inhabitants of Middle America as related to causation of human and animal disease. Special emphasis is placed on arthropod-borne viruses, their natural reservoirs and vectors, as well as other agents which may be of potential danger to the populations of other American countries, including the United States.

Methods Employed:

Elucidate the significance of finding viruses in the tropical environment by attempted identification of the agents, definition of their natural distribution and reservoirs, possible vectors, and rates of infection as reflected by the frequency of isolation and the presence of specific antibodies in the general population or cases of recognizable illness.

Virus isolations are attempted in animals, embryonated eggs, and cell cultures of monkey and hamster kidney, chick embryo, human amnion, and other cell types. The cultures are observed for CPE and some are subjected to hemadsorption and hemagglutination diagnostic tests. Serological tests and identification of fresh isolates employ virus neutralization procedures in tissue culture and mice, hemagglutination and hemadsorption inhibition, and complement fixation.

Diagnostic materials regularly utilized for isolation and/or serological testing included specimens of blood (human, other mammalian, avian, and reptilian), cerebrospinal fluid, throat and rectal swabs, autopsy specimens, and mosquito pools.

Major Findings:

A field study of an outbreak of eastern equine encephalomyelitis in horses in the Republic of Panama has been attempted. From the Pacora area where cases of encephalitis in horses have been observed, numerous specimens have been collected for laboratory testing. For serological testing, sera from over 200 wild birds, about 100 wild bats, 55 human beings, and 65 horses have been collected. In addition a total of 2,500 human sera from the Republic of Panama have been collected for similar testing. Materials from most of the animals and birds were collected for the purpose of attempting viral isolation as well as more than 25,000 mosquitoes of at least 11 different genera. No isolation of EEE virus has been made from any source except the brains from the two encephalitic horses. Serological studies in progress so far disclosed antibodies against this virus in 40 of the 65 horses, possibly in three of the humans, and in none of the domestic fowl tested. The initial results suggest that although it may not be possible to establish conclusively either the vector or the reservoir of EEE in Panama the epidemiological and ecological concepts worked out for the Eastern United States indeed do not apply to the tropics.

Other viral studies have been carried out in the Middle America Research Unit laboratories on influenza, poliomyelitis, epidemic

hemadsorption virus infection, and numerous viral diagnostic procedures have been carried out for hospitals of Middle American countries. The most significant studies and findings of the MARU are itemized and described briefly below:

The earliest laboratory procedure performed at MARU was an examination of 50 paired sera submitted by Gorgas Memorial Laboratory from patients seen at Gorgas Hospital with suspected Asian influenza during August and September 1957. In January 1958 the sera were tested by the MARU staff: of the 50, 13 had four-fold or higher rise in specific antibodies, 15 had a rise from <10 to 20, and 22 showed no antibody rise.

Twelve rectal swabs were procured in August from Managua paralytic cases through the courtesy of Maj. R. Singer, Surgeons' Office, USARCARIB. Although each swab was inoculated into a single MK TC tube, 10 of the 12 swabs produced typical polio-like CPE. Three representative strains were typed at MARU as poliovirus type 2. Comparison of results with Dr. John R. Paul's data on 35 Managua patients disclosed that 7 of the same patient specimens were also included in the Yale sampling and were found to contain polio 2 virus. These findings were important because the Nicaraguan outbreak was the first epidemic of any size which could be attributed to type 2 polio.

In April, May, and June 1958, during a prolonged outbreak of severe respiratory illness, 3 strains of a possibly new viral agent were isolated in human amnion TC by the use of hemadsorption techniques developed at NIH in 1957 by the present members of MARU staff. In certain respects the agent seemingly differed from the hemadsorption virus types 1 and 2 recently defined by Channock et al. at NIH. Its precise relation to the other members of the HA virus group is under study. All 3 patients suffered from a systemic febrile illness but only 2 of them exhibited significant rises in antibody levels. Preliminary check of random sera collected in July from young adults in the terminal cities of Panama and Colon, on the Pacific and Atlantic side of the Isthmus respectively, showed a possibly significant difference in antibodies demonstrable by hemadsorption-neutralization test: 15/30 on the Pacific side had significant levels, but only 1/10 on the Atlantic side seemed to be immune.

Young pediatric ambulatory patients with diarrhea (100), with severe respiratory symptoms (100), and with undifferentiated febrile illnesses (50), were sampled by collecting both rectal and throat swabs from each patient. Unfortunately, the urgency of the EEE epizootic studies delayed actual testing of the materials collected. It is anticipated that common respiratory and enteric viruses will be demonstrated in most of the positive specimens, but a possibility

exists that new fundamental information will be obtained warranting a detailed inquiry at a later date.

A surprisingly large number of recent paralytic cases have been variously recorded by physicians as atypical polio, Guillain-Barre Syndrome, infectious neuronitis, and so forth. Viral isolations attempts have been unsuccessful and serological studies disclosed no antibody rises against polioviruses. Further studies are indicated.

Significance to the Program of the Institute:

Recent reawakening of general interest in investigations of tropical infectious diseases through application of a newer virological and mycological tools was anticipated by the early plans for the establishment of MARU as an interservice organization, initially sponsored by the National Institutes of Health and the Walter Reed Army Institute of Research. In spite of its brief existence, with much of the time devoted to organization of the physical facilities and laboratory techniques, interesting data of concern to both disciplines were collected.

The most important phase of the virological activities was the field study of the EEE epizootic in Panama in collaboration with the Gorgas Memorial Laboratory and the Laboratorio Veterinario. Although this investigation at the time of this writing is still only at about half-way mark, results are accumulating to indicate that a rather different ecological situation in relation to EEE virus prevails in Central America as compared to the Eastern U.S. Investigations of other locally isolated ARBOR viruses, besides yellow fever which is under study at Gorgas Memorial Laboratory, have been begun to determine their significance in relation to public health problems.

A study of specimens collected from non-hospitalized pediatric clinic patients should provide preliminary information on the nature and prevalence of local enteric and respiratory viruses.

A limited study of the specimens from the polio outbreak in Nicaragua provided the early evidence that this was indeed a remarkable type 2 poliovirus epidemic.

Continued examination of clinical specimens from patients with assorted febrile illnesses in the C. Z. and the R. de P. (especially from cases of febrile paralytic disease and aseptic meningitis syndrome) is already elucidating the etiologic nature of these clinical problems and offering clues for additional research ideas and subprojects.

Proposed Course of the Project:

Most of the unfinished studies discussed will be continued to completion. It is anticipated that about 65 to 75% of total time will be expended on ARBOR virus work and, depending on circumstances and opportunities, the remainder on the non-ARBOR agents.

It is hoped to devote time to development of better field laboratory tools in collaboration with the Tropical Arbor Virus Section, LVD, in Bethesda, such as optimal condition for TC cultivation and for hemadsorption techniques with ARBOR viruses, simplification of complex ARBOR serological procedures for rapid survey work, and utilization of locally available monkeys as a source of renal tissue.

A new research interest will be an evaluation of the Group C of ARBOR viruses in etiology of clinical disease in Central America.

The major endeavor contemplated for the new year is a comprehensive collaborative field study in the Bocas del Toro province to be operated jointly with the Gorgas Memorial Laboratory. The study is visualized as a long range accumulation of virological, entomological, ecological, and epidemiological data by the personnel of a permanent field station to be located in the tropical rain forest of the Republic's Atlantic coast, which abounds in a variety of mammals, birds, reptiles, and amphibians, as well as arthropods. The combination of this laboratory's virological facilities and skills with the unequaled entomological and tropical field experience of the Gorgas Memorial group is bound to result in a significant contribution to our knowledge of arthropod transmitted viruses in Middle America.

Part B included - No

Serial No. NIAID-125-B
1. Tropical Diseases
2. Middle America Research
3. Guatemala, C. A.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on arthropod-borne viral diseases in Guatemala

Principal Investigator: Charles G. Dobrovolny

Other Investigators: None

Cooperating Units: Pan-American Sanitary Bureau
Ministry of Health, Guatemala

Man Years (calendar year 1958):

Total:	2 1/4
Professional:	1
Other:	1 1/4

Project Description:

Objectives:

To determine which tropical arthropod-borne viruses are present and active in Guatemala and to assess their importance as a cause of disease of human beings in Guatemala.

Methods Employed:

In collaboration with the Pan-American Sanitary Bureau and the Ministry of Health of Guatemala, specimens are collected from selected human beings and animals in Guatemala. These specimens consist of sera from selected healthy population groups in Guatemala and specimens from patients in the acute phase of diseases suspected of being caused by tropical arthropod-borne viruses. These specimens are submitted to the Section on Tropical Arthropod-borne Viral Diseases for study.

Major Findings:

See the report of the Section on Tropical Arthropod-Borne Viral

Diseases for results of tests on sera collected in Guatemala. During the period of collection in Guatemala, about 600 sera were collected from human beings in representative locations throughout the country.

Significance to the Program of the Institute:

Determinations of the arthropod-borne viral agents existing in Guatemala which are of importance as a cause of disease will indicate the most valuable directions of investigations of tropical arthropod-borne virus diseases in Central America.

Proposed Course of the Project:

This project will be terminated in Guatemala during the coming year and the type of study made in Guatemala will be continued in the Republic of Panama.

Part B included - No.

Serial No. NIAID-125-C
1. Tropical Diseases
2. Middle America Research Unit
3. Ancon, C. Z.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Studies of histoplasmosis on the Isthmus of Panama

Principal Investigator: Robert L. Taylor

Other Investigators: Charlotte Campbell

Cooperating Units: Walter Reed Army Institute of Research
Gorgas Hospital
Gorgas Memorial Laboratory
Ministry of Health
Health Director, C. Z.
Coco Solo Hospital, C. Z.
Army, Navy, and Air Force Surgeons, Caribbean area
U.S. Army Preventive Medicine Officer, USARCARIB
U.S. Malaria Control and Survey Branch, Corozal, C. Z.
Military Sea Transport Service

Man Years (calendar year 1958):

Total:	3 1/6
Professional:	1
Other:	2 1/6

Project Description:

Objectives:

To determine the prevalence and epidemiological foci of histoplasmosis on the Isthmus of Panama. Initiate cooperative mycological studies with interested investigators in Middle America countries.

Methods Employed:

Obtain initial data on previous experience with H. capsulatum for individuals arriving in the Isthmus of Panama. Carry out periodic skin test checks on individuals thereafter to determine if individual has become hypersensitive to extract of organism (histoplasmin). Carry out chest X-ray determinations on all persons converting to hypersensitivity.

In clinical cases of suspect histoplasmosis, carry out diagnostic procedures. Carry out tests for organism on samples of soil obtained in various areas in Isthmus of Panama and test indigenous animals for presence of organism or for serological evidence of association with the histoplasma organism.

Major Findings:

Since the initiation of investigations in May 1958, the existence of acute histoplasmosis on the Isthmus of Panama has definitely been established. In cooperation with the Isthmian medical facilities over thirty cases of histoplasmosis have been serologically confirmed. Attempts to recover H. capsulatum from acute cases of histoplasmosis have been unsuccessful as was to be expected from the experience of previous investigators. The animal studies indicate that spiny rats, which comprise 80 per cent of trapped ground mammals in Panama, can be infected with H. capsulatum and are undoubtedly infected in nature. Spiny rats collected locally have been found to possess complement-fixing antibodies and skin hypersensitivity to histoplasmin. Preliminary investigations of the local bats indicate that they also represent a potential reservoir of histoplasmosis.

Soil samples collected in Panama and processed by Miss Campbell at WRAIR, have yielded two isolations of H. capsulatum. One isolation being from soil collected in a forest preserve in the Canal Zone and the other from a soil collected in the Republic at Cerro Azul.

An estimate of the prevalence of histoplasmosis on the Isthmus must await the results of an extensive skin testing program which is currently in progress. More than 500 newly arriving individuals have been skin tested with histoplasmin, an experimental histoplasmin, and tuberculin. The first results of retesting the non-hypersensitive individuals will be forthcoming in 1959. Initial testing indicates approximately one third are hypersensitive upon arrival.

Foci of infection must await further investigations of the "leads" from histories obtained from hospitalized cases of histoplasmosis.

Significance to the Program of the Institute:

Information on the true importance of histoplasmosis on the Isthmus of Panama in newly transplanted persons is lacking. The disease was unrecognized for approximately 45 years and now with careful vigilance the significance of histoplasmosis in fever of unknown etiology is again being appreciated, particularly in U. S. military personnel and civilians residing in the area. It is extremely important to define the significance of this infection as a

major health problem in the Republic of Panama.

Proposed Course of the Project:

The Project will be pursued until satisfactory evaluation of histoplasmosis as a health problem in Panama has been accomplished.

Histoplasmin skin testing to determine the extent of histoplasmosis in the area and serologic and cultural techniques to determine the foci of infection will continue to be utilized.

Efforts are being made to establish an army unit consisting of the Army personnel assigned to MARU. This will provide a means of expanding the military and civilian personnel on the staff and to incorporate the mycology research group into the Caribbean Army Command.

Part B included - No

Serial No. NIAID-126-A
1. Tropical Diseases
2. Section on Epidemiology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of the exoerythrocytic phases of primate malaria

Principal Investigator: Don E. Eyles

Other Investigators: Robert L. Ingram, Nell C. Owen

Cooperating Units: None

Man Years (Calendar year 1958):

Total:	3 3/4
Professional:	2
Other:	1 3/4

Project Description:

Objectives:

To determine if the reported occurrence of exoerythrocytic parasites of primate malaria in the liver is correct. To extend the knowledge of the exoerythrocytic stages, in particular to trace the development for sporozoite to the initiation of the parasitemia and to determine the origin and nature of the late tissue stages which are apparently related to relapse. To develop techniques for obtaining massively infected tissues for in vitro study. To define fully quantitative relationships in this cycle, the study of which has been largely qualitative up to this time. To determine the nature of the action of drugs in vivo on established exoerythrocytic infections.

Methods Employed:

Plasmodium cynomolgi and other primate malarias are maintained in Macaca mulata and other monkeys. An insectary is maintained to supply mosquitoes for passage of the malaria. Mosquitoes are infected in large numbers. Salivary glands are dissected and injected intravenously in order to produce massive exoerythrocytic infections.

At selected intervals after inoculation, the livers of monkeys are taken by laparotomy. Sections are prepared, stained, and examined to observe the nature of the infection. Observations are also made of live material. Radioactivity tagging and/or flourescent antibody technique shall be used in following the parasite during early stages which are difficult to follow morphologically. Other cytochemical methods shall be used as needed.

Human malaria material is used as available from the Columbia (S. C.) laboratory which is cooperating in this study.

Major Findings:

Very thorough control work has indicated that inoculations of as many as 2,026 uninfected mosquitoes do not produce structures similar to described exoerythrocytic stages; nor do uninfected monkeys show such bodies naturally. Whenever salivary glands from eight or more mosquitoes have been injected, light to heavy infections of the liver with exoerythrocytic parasites have resulted. Furthermore, many animals have shown such parasites at the occasion of the first biopsy, ruling out the possibility that previous surgical intervention could have caused artefacts.

The exoerythrocytic infections have been quantitated, and it has been found that the number is positively correlated with the number of infected glands injected. Likewise there is a very strong positive correlation between the number of exoerythrocytic forms and the resulting parasitemia. Just at the time the parasitemia develops, the number of exoerythrocytic forms falls sharply. During the period between primary attack and the relapse, the number of exoerythrotic forms is low, but parasites can be found then and also at the time relapse is expected.

The parasite has been followed from the fourth through the 120th day after inoculation. From day four through nine the parasites in the liver cells grow greatly. Maturity occurs between day eight and nine and blood invasion occurs then. Parasites seen from 10 to 120 days after inoculation have mostly been large, thus leaving uncertainty as to the occurrence of secondary exoerythrocytic cycles.

Treatment of monkeys given massive injections of infected material with primaquine and pyrimethamine has resulted in apparently complete clearance of the liver of parasites. Pyrimethamine-treated monkeys develop infections about 40 days in spite of this

clearance, which opens a question as to how a few parasites survive, though most are certainly destroyed.

For use in in vitro studies, it has been possible to obtain infections in the liver approaching 50,000 per milliliter.

Significance to the Program of the Institute:

The work accomplished to date removes any doubts that exoerythrocytic stages of primate malaria do occur in the liver. It contributes also to the knowledge of these, particularly as regards quantitative relationship.

Since effective chemotherapy of malaria depends upon the removal of the exoerythrocytic stages, a knowledge not only of the morphology but also of the physiology of these parasites is essential as a basis for understanding the action of drugs. This phase of the life cycle of malaria is the least well known and this project, along with its coordinated project of in vitro study, may well produce additional essential fundamental information. Observations so far have given rise to new problems with both fundamental and practical applications to chemotherapy.

Proposed Course of Project:

The project shall continue to explore the exoerythrocytic cycle as intensively as possible. The in vivo studies shall be closely correlated with the in vitro studies, so as to make maximum utilization of materials. More emphasis shall be given to cytochemical and physiological studies as the experiments progress.

Part B included - No.

Serial No. NIAID-126-B
1. Tropical Diseases
2. Section on Epidemiology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of the in vitro growth of primate malaria parasites.

Principal investigator: Robert L. Ingram

Other Investigators: Jon E. Eyles and Frances E. Jones

Cooperating Units: None

Man Years (Calendar Year 1958):

Total:	3
Professional:	1 3/4
Other:	1 1/4

Project Description:

Objectives:

To devise methods of maintaining primate malaria parasites in vitro, either in growing cell cultures or in organized tissue maintained in vitro. To study at the molecular level the physiology of the malaria parasite, the pharmacological action of chemotherapeutic agents on the prasite, and the effect of the parasite on the host cell metabolism.

Methods employed:

Standard cell and organ culture methods are employed being modified when necessary. Material for culture is obtained from the closely coordinated project on the study of exoerythrocytic parasites. Appropriate biochemical methods are used in metabolic studies of both parasitized and unparasitized cells.

Major Findings

Cell lines have been established from monkey liver and other sources. Some success has been achieved in maintaining organized liver tissue in vitro. A number of experiments involving the inoculation of cultures with malaria sporozoites have been done, but evaluation is incomplete.

Infected liver tissue has been maintained in vitro but evaluation of the results is also incomplete.

Significance to the Program of the Institute:

The use of in vitro methods to study the malaria parasite, particularly the exoerythrocytic stages, will make possible studies of a molecular level of the physiology of the parasite. Basic information may be obtained on the action of antimalarial drugs. Information developed may be applicable to other parasitic infections of man and animals. Metabolic studies of cell in tissue culture either parasitized or unparasitized, may provide new information on cellular functions.

Proposed Course of the Project.

The project is now in its earliest stages. The effort during the coming year will be directed toward the establishment of cell lines and the growth of parasites in these cell lines, and toward development of methods of maintaining infected liver tissue in vitro. Studies of cellular function in cell culture and organ culture will be made. Once practical methods of maintaining the parasite in vitro are developed, the effort will be concentrated on biochemical studies of the physiology of the parasite and the pharmacological action of drugs upon the parasite.

Some attempts may be made to culture insect tissues in order to study aspects of the parasite in the insect host. In vivo as well as in vitro studies will be made on the pharmacology of drug action to determine metabolites of the drugs, enzymes affected, etc., this to be coordinated closely with the project on the study of the exoerythrocytic stages of primate malarias.

Part B included - No.

Serial No. NIAID-126-C
1. Tropical Diseases
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Studies on human malaria

Principal Investigator: Martin D. Young

Other Investigators: Robert W. Burgess and Aimee Wilcox

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1958):

Total:	4 2/3
Professional:	1 2/3
Other:	3

Project Description:

Objectives:

To add to the biological knowledge of malaria by observations on induced malaria and experimental studies. To determine the sporontocidal activity of antimalarial drugs and how developed resistance influences transmission by mosquito vectors. To study the development and inheritance of resistance to drugs by the parasites. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for the therapy of neurosyphilis, nephrosis, Parkinsonism, and for experimental malaria studies.

Methods Employed:

Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. The patent infections are challenged by drugs. When resistance occurs measurements are made of its characteristics. Mosquitoes are allowed to bite patients with induced infections before and at intervals after drug administration. The resulting infections in the mosquitoes are correlated with the presence or absence of drug resistance of the malaria infection in the human host.

Major Findings:

Pyrimethamine resistance by Plasmodium falciparum infections developed rapidly after an initial dose of 25 mgm., being manifested during relapses in the second and subsequent challenges by the drug. Resistance was evidenced by the loss of schizontocidal and sporontocidal effects. Increasing the drug dosage did not overcome the resistance. The resistance factor was transmitted, apparently undiminished, by mosquitoes.

Similarly, resistance by P. vivax developed after initial doses of either 25- or 100 mgm. doses of pyrimethamine. The second and subsequent treatments with the same or higher doses were not effective on either the asexual or sexual parasites. The resistant quality persisted undiminished in subsequent infections transmitted by bites of mosquitoes, blood transfusion, or the injection of sporozoites which had been preserved at low temperatures.

There appears to be no cross resistance between pyrimethamine and Camoquine or primaquine.

The sporontocidal effect of a 30 mgm. single dose of primaquine was demonstrated within 12 hours against P. vivax and 48 hours against P. falciparum. Primaquine given 3 mgm. daily, 15 mgm. twice weekly, or 30 mgm. once weekly, exerted continuous sporontocidal effects, starting several days after the first dose, upon patent P. vivax and P. falciparum infections. Against P. vivax, 5 mgm. daily had a similar effect. P. vivax responded quicker than P. falciparum. These dosages exerted a slow schizontocidal effect in most cases.

Blood parasites and sporozoites have been preserved successfully for periods up to four years at low temperatures.

Significance to the Program of the Institute:

The development of pyrimethamine resistance by human malaria parasites indicates the need of basic research on the biology of the human malaria parasite. The demonstration of sporontocidal action of small frequent doses of primaquine suggests experimental chemotherapeutic trials to determine the practicality of such use in malaria eradication programs.

Proposed Course of the Project:

To develop an experimental physiological investigation to determine the mode of action of drugs on human malaria parasites in both man and mosquito, how resistance to drugs is developed and how it may

be avoided. The factors which make vectors hospitable to parasites will be sought both by in vivo and in vitro methods. Cooperative projects will investigate the prophylactic and supressive action of regular doses of antimalarial drugs, such as primaquine, with the aim of practical use in malaria eradication programs.

Part B included - Yes

Serial No. NIAID-126-C

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Jeffery, G. M. Infectivity to mosquitoes of Plasmodium vivax following treatment with Chloroquine and other antimalarials. Am. J. Trop. Med. and Hyg. 7: 207-211, 1958

Coatney, G. R., Mickelsen, O., Burgess, R. W., Young, M. D., and Pirkle, C. I. Chloroquine or pyrimethamine in salt as a suppressive against sporozoite-induced vivax malaria (Chesson strain). Bull. Wld. Hlth. Org. 19: 53-67, 1958.

Jeffery, G. M. Book Review: Bray's Studies on the Exo-Erythrocitic Cycle in the Genus Plasmodium. Am. J. Trop. Med. and Hyg. 7: 575-576, 1958.

Young, M. D. and Burgess, R. W. Pyrimethamine resistance in Plasmodium vivax malaria. Bull. Wld. Hlth. Org. (In press).

Burgess, R. W. and Young, M. D. The development of pyrimethamine resistance by Plasmodium falciparum. Bull. Wld. Hlth. Org. (In press).

Young, M. D. Chemotherapeutic agents and malaria eradication. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In press).

Jeffery, G. M. Early activity in sporozoite-induced Plasmodium falciparum infections. Annals of Trop. Med. and Parasit. (In press).

Garnham, P. C. C., Jeffery, G. M. and Young, M. D. Preservation of strains of malaria parasites. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In press).

Honors and Awards relating to this project:

For Dr. Martin D. Young:

Member, Expert Advisory Panel on Malaria, World Health Organization.
Chairman, Editorial Board, American Journal of Tropical Medicine and Hygiene.
Co-Chairman, Section on Clinical Malaria. Sixth International Congresses on Tropical Medicine and Malaria.
Delegate from American Society of Parasitology to Sixth International Congresses on Tropical Medicine and Malaria.
Charter Fellow, American Academy of Microbiology.

Serial No. NIAID-126-D
1. Tropical Diseases
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chemotherapy of intestinal parasites in a mental institution

Principal Investigator: Martin D. Young

Other Investigator: Geoffrey M. Jeffery

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1958)

Total:	3¼
Professional:	1
Other:	2¼

Project Description:

Objectives:

To find adequate drugs for the prophylaxis and treatment of parasitic infections which constitute serious health problems in certain types of mental patients as well as in more normal populations; to determine the relationship of parasite load to psychological or psychiatric considerations in the patient; also to investigate factors influencing parasite spread and persistence among the patients.

Methods Employed:

Promising compounds in varying dosages are tried against parasitic infections. Selected segments of the population are treated with the most effective drugs available to determine the possible alteration of physical and mental condition in the host by the removal of parasite infestation. Careful pre- and posttreatment psychological, psychiatric and physical evaluations are done.

Major Findings:

Extensive trials of a new drug, bephenium, have been done, representing the first major controlled investigation of the drug against human hookworm. Results have been encouraging. Three salts have been tested: the chloride, the bromide, and the hydroxynaphthoate. The more effective regimens have produced up to 85 per cent reduction in worm burden. Trials with the new anthelmintic drug, dithiazanine iodide have confirmed the effectiveness of this drug against Trichuris. Results of treatment of hookworm with dithiazanine have been disappointing.

The degree of parasitism in mental patients was correlated with psychological and psychiatric tests. It was found that the heavier parasitic infections were correlated with poorer psychological reactions as indicated by hospital adjustment scores. Upon removal of most of the parasites by treatment, the physical findings, i.e., hemoglobin, lower eosinophilic densities, etc., were improved, but there was no significant improvement in the results of psychological tests.

Epidemiological studies have shown a high percentage of persistence of Trichuris and hookworm in a group of patients for more than four years. Among the protozoa only with Entameba coli did significant numbers of infections persist for this period.

Significance to the Program of the Institute:

Certain types of institutions, such as mental hospitals, are reservoirs of parasitic infections and some of the occupants are adversely affected by heavy worm infections. The finding of prophylactic and curative drugs for these infections is important not only for institutions, but for normal populations where parasitism is a health problem. The possibility of improving the adjustment of the mental patient by removal of worm infections is of importance.

Proposed course of the Project:

The screening of promising compounds in the search for better parasitocidal drugs will be continued. Various dosages and regimens of the newly found effective drugs against hookworm and Trichuris will be tried in an effort to produce better means for treatment and prophylaxis of these infections in mental institutions and in more normal groups. Further investigations on the relationship of parasite load to psychological, psychiatric and physical considerations in the patients will be continued.

Part B included: Yes

Serial No. NIAID-126-D

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Disanaike, G. A., Jeffery, G. M., and Barton, B. P. Radioactive tagging of hookworm larvae (Necator americanus) with P^{32}. Exp. Parasitol. 7: 249-253. 1958.

Jeffery, G. M. Epidemiologic considerations of isosporiasis in a school for mental defectives. Am. J. Hyg. 67: 251-255. 1958.

Young, M. D., Jeffery, G. M., Freed, J. E., and Morehouse, W. G. Bephenium, a new drug active against human hookworm. J. Parasitol. (In press.)

Young, M. D., Jeffery, G. M., Freed, J. E., and Morehouse, W. G. The effectiveness of dithiazanine against worm infections in mental patients. Archives of Neurology and Psychiatry. 80: 785-787.

Honors and Awards relating to this project: None

Serial No. NIAID-126-E
1. Tropical Diseases
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Virus-Parasite Associations

Principal Investigator: Geoffrey M. Jeffery

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1/2
Other:	1 1/2

Project Description:

Objectives:

To determine if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. If evidence establishes such relationships between parasites and viruses, further objectives are to study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships. This includes the study of possible intestinal viruses among institutionalized mental patients. To investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. To investigate the effects of parasitic infections upon virus infections and vice versa.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as LCM, polio, St. Louis encephalitis, rabbit papilloma and fibroma are maintained in small animals by serial transmission, providing infective virus material for experimental work. Viruses and parasites are brought together in various ways, such as by simultaneous infection in animals, during cultivation of the free-living stages, or by simultaneous exposure of the host to infective

parasite stages and virus suspensions. Tissue culture or small animals are used for detection of virus infection carried by parasites from virus-infected animals, for virus detection in parasites suspected of harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients is studied with animal inoculation, tissue culture, and other means.

Major Findings:

Further studies confirmed the results of the prior year where simultaneous infection of mice with Nippostrongylus muris and SLE virus gave rise to N. muris larvae from which virus could be isolated. These results were also repeated using LCM virus and N. muris in mice. Using another nematode parasite, Nematospiroides dubius, limited studies have shown the same phenomenon with both the LCM and SLE viruses. All of these studies have indicated that there is viral multiplication within the parasite after the parasite has been deposited by the host and while it is awaiting entry to a new host, i.e., during development to the infective stage. An interesting finding was in the increased number of deaths in mice receiving N. dubius and LCM virus simultaneously than was seen with intragastrically either pathogen alone. Undoubtedly this indicates a mechanical introduction of virus along with larval penetration in the gut.

Significance to the Program of the Institute:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theoretical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of association of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produced by such organisms, alone or in combination.

Proposed Course of Project:

The general course of the project will be maintained. Repetition and expansion of the experiments combining nematode parasites and viral encephalitides in the vertebrate host, during free-living stages and in culture, will constitute the major program. Further refinement of viral detection methods will be investigated and incorporated in the work when practicable. Whereas most of the experimental work to date and planned is in the realm of artificial viral-parasite associations, the possibility of demonstration of naturally occurring associations will always be an objective. Viral outbreaks in heavily parasitized groups in the institutional populations will be explored for evidence of such associations.

Part B included - Yes

Serial No. NIAID-126-E

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project:

Dr. G. M. Jeffery was named Editor of the Bulletin of the Association of Southeastern Biologists.

Serial No. NIAID-126-F
1. Tropical Diseases
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cytological studies of parasites

Principal Investigator: Gordon B. Wolcott

Other Investigators: None

Cooperating Units: None .

Man Years (calendar year 1958):

Total:	2/3
Professional:	2/3
Other:	0

Project Description:

Objectives:

To study chromosome number, morphology, and behavior in parasites, with emphasis on human parasites, or closely related forms. To locate, when possible, the point in the life cycle, especially of protozoan parasites, at which meiosis occurs in order to lay a basis for future genetic studies.

Methods Employed:

The nuclei of parasites are studied in fixed and stained preparations, including the use of cytochemical tests, and in the living condition under bright field and phase contrast microscopes. All available stages of the life cycle are examined, under normal and experimental conditions.

Major Findings:

The preliminary report that the chromosome number of Diphyllobothrium ursi is N = 9, 2N = 18, was confirmed. Four of the paired chromosomes are very small. Phase contrast microscopy and cinephotomicrography added significant evidence that malaria parasites are inside the erythrocytes.

Significance to the Program of the Institute:

So little is known of the cytology or genetics of human parasites that any information will be of value. It is hoped that eventually the basic cytological information being collected in this work will lead to a logical study of inheritance of physiological and morphological characteristics of human parasites. The determination of the chromosome patterns of species of Diphyllobothrium may lead to a more exact classification of this taxonomically difficult group of organisms, some of which are important as human parasites, or as zoonoses, especially in Alaska where the infection rate in some areas is high. Cytological studies may aid in determining the number of species, now unknown, actually existing in that country.

Proposed course of the Project:

This work has been discontinued as the cytologist left to take a teaching position.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Wolcott, G. B., Young, M. D., and Ferguson, M. S. On the position of Malarial Parasites with Relation to Erythrocytes. Trans. Roy. Soc. Trop. Med. and Hyg. 52: 87-90. 1958.

Wolcott, G. B. The Chromosomes of Diphyllobothrium ursi, J. Parasit. (In press)

Honors and Awards relating to this project: None

Serial No. NIAID-127-A
1. Tropical Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Studies on toxoplasmosis

Principal Investigator: Leon Jacobs

Other Investigators: Jack S. Remington, Marjorie L. Melton, Milford N. Lunde, Anastasia S. Stanley

Cooperating Units: Ophthalmology Branch, NINDB - 69 (c)

Man Years (calendar 1958):

Total:	6
Professional:	4
Other:	2

Project Description:

Objectives:

The elucidation of the epidemiology of toxoplasmosis in man and animals, and of the role of various animal reservoirs in transmission to humans; the understanding of the relationship of the proliferative and encysted stages of the parasite, in transmission and in the production of chronic disease; the evaluation of the usefulness of serological tests for the diagnosis of toxoplasmosis; the determination of the identity of the antibodies demonstrated in the various tests; the description of factors related to the virulence of Toxoplasma, and of those involved in the susceptibility and immunity of the host, especially in regard to exacerbations of chronic infection and to the possibility of the occurrence of congenital transmission from chronically infected mothers; the establishment of an experimentally determined basis for the therapy of toxoplasmosis; the study of the parasite in vitro, especially in regard to physiological factors related to virulence, drug action, and immunity; the description of the various forms of symptomatic toxoplasmosis.

Methods employed:

The techniques are standard procedures such as are used in virology, including the range of animal inoculations and such additional

methods as injections into the anterior and posterior chambers of the eye. A micro-isolation apparatus is employed in working with encysted forms. Tissue cultures are used. The dye test and hemagglutination test are used for serological studies, and the antigens and antibodies are investigated by biochemical and electrophoretic techniques.

Major Findings:

Toxoplasma has been demonstrated in 8 of 66 samples of skeletal muscle from sheep slaughtered in Baltimore.

The ability of Toxoplasma cysts to withstand digestion, heat, and osmotic pressure changes has been tested. The cyst wall is immediately digested by pepsin or trypsin; the liberated parasites can survive briefly in pepsin and longer in trypsin. Cysts are rendered non-infective by brief heating or immediately on being placed in distilled water.

Congenital transmission of toxoplasmosis from chronically infected mother rats to their offspring has been found to occur only rarely. It occurs more frequently in mice infected with a cyst-producing strain of Toxoplasma. Residual infection in the uterus has been demonstrated in chronically infected rats and mice, suggesting a means for congenital transmission without a generalized exacerbation in the mother.

In a study of residual infection in human uteri, Toxoplasma cysts were demonstrated in the uterus of a woman with serological evidence of past infection.

The rate of lysis of tissue cultures infected with various Toxoplasma strains is directly related to their invasiveness and growth rate, and to their virulence in animals. Slow-growing strains are less susceptible to pyrimethamine than more rapidly growing parasites. Slow-growing strains can produce "chronic infection" in tissue cultures.

The identity of neutralizing and dye test antibodies has been established. This is an important point in relation to the specificity of the dye test and to immunological phenomena observed in toxoplasmosis.

Detailed information has been gathered on the stability of the hemagglutination test antigen in the liquid and solid states at various temperatures. Powdered antigens are stable at high temperatures. In acute toxoplasmosis, the hemagglutination test reveals antibodies about 4 days later than the dye test; there is evidence that this may be due to an inhibiting substance in the acute serum, rather than to different antibodies. Eventually hemagglutinating titers reach the same levels as those found by the dye test. Hemagglutination-inhibiting substances are found in some small animal sera.

Bio-assays of pyrimethamine in the serum of rabbits following one dose of 100 mg/kg showed decreasing levels up to 7 hours. Pyrimethamine was detectible in the retina of a monkey. Also, in guinea pigs treated with pyrimethamine and sulfa following inoculation of Toxoplasma into the posterior chamber of the eye, chorioretinitis did not develop, and parasites were only rarely recoverable from the ocular tissues.

Significance to the Program of the Institute:

Knowledge of the means of spread of Toxoplasma is of great importance as a basis for recommendations for prevention of the infection. At the present time, advocacy of the thorough cooking of meat could conceivably be effective in preventing cases of congenital toxoplasmosis and of toxoplasmic chorioretinitis. Further work may elucidate the cycle in herbivorous animals, to allow some control there.

The development of the hemagglutination test for toxoplasmosis has been motivated by the need for a substitute for the dye test, which is too impracticable for routine use. Except for the slight difference in time of demonstration of antibodies in the acute infection, the hemagglutination test appears to fulfill the need. Furthermore, it may serve better than the dye test for some studies on domestic animals, whose sera react peculiarly in the dye test.

The factors which are involved in alterations in host-parasite balance in chronic Toxoplasma infections, formation and rupture of cysts, and in susceptibility and immunity of hosts, invasiveness and pathogenesis of strains, host-parasite relations at the cellular level, are all subjects for basic study. Investigation of these problems can be expected to furnish explanations for clinical observations and also to contribute to understanding of other infections in which chronicity is observed.

The demonstration of congenital transmission from chronically infected mother animals is of considerable importance in relation to the occurrence of various types of neonatal disease.

The importance of an understanding of chronic infection is fundamental to the management of ocular toxoplasmosis, and to any studies which may be possible on the relation of Toxoplasma to obscure myocarditis.

Toxoplasma is an excellent organism for study from the standpoint of the basic problems of humoral and cellular immunity, and the hypersensitivity factor in pathogenesis.

Proposed Course of Project:

Attention will be devoted to the following points during the next calendar year; (1) The epidemiology of toxoplasmosis in herbivorous animals, such as sheep and cattle; (2) the possible occurrence of an intermediate stage in invertebrate hosts; (3) the usefulness of the hemagglutination test in the study of infections in domestic animals; (4) continued exploitation of the hemagglutination test in patients with ocular disease, especially in regard to titers found in such individuals as compared to "normals"; (5) study of the biology of cyst-formation and of the possible occurrence of unencysted dormant parasites in chronic infections; (6) the relation of cysts and dormant parasites, if any, to recurrent disease, and the definition of factors involved in such disease, especially in the eye and the heart; (7) the relation of antibodies to persistence of organisms in various tissues, of chronic infections; (8) the effect of artificially produced high levels of antibody on cysts and on congenital transmission from chronic infections; (9) the effect of drugs in chronic infections; (10) the relationship of dye test and hemagglutinating antibodies; (11) the relation of local tissue immunity to the spread of challenge infections; (12) continuation of study of serial dye test titers on normal individuals to obtain a base line for evaluating fluctuation in antibody titers seen in cases of presumed ocular toxoplasmosis.

Part B included - Yes

Serial No. NIAID-127-A

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Jacobs, Leon and Lunde, Milford N.: A hemagglutination test for toxoplasmosis. J. Parasitol. 43, 308-314, 1957 (omitted from 1957 report)

Cook, M. Katherine and Jacobs, Leon: Cultivation of Toxoplasma gondii in tissue cultures of various derivations. J. Parasitol. 44, 172-182, 1958.

Cook, M. Katherine and Jacobs, Leon: In vitro investigations on the action of pyrimethamine against Toxoplasma gondii. J. Parasitol. 44, 280-288, 1958.

Cook, M. Katherine: The inhibitory effect of adenine and related compounds on the proliferation of Toxoplasma gondii in tissue culture. J. Parasitol. 44, 274-279, 1958.

Cook, M. Katherine and Jacobs, Leon: Storage of pyrimethamine in cells in vitro. Proc. Soc. Exper. Biol. Med. 98, 195-198, 1958.

Cook, M. Katherine: The development of a pyrimethamine-resistant line of Toxoplasma under in vitro conditions. Am. J. Trop. Med. Hyg. 7, 400-402, 1958.

Remington, Jack S.; Jacobs, Leon; and Kaufman, Herbert E.: Studies on chronic toxoplasmosis. The relation of infective dose to residual infection and to the possibility of congeni[illegible]smission. Am. J. Ophthalmol., 46, 261-267, 1958.

Kaufman, Herbert E.; Remington, Jack S.; and Jacobs, Leon: Toxoplasmosis: the nature of virulence. Am. J. Ophthalmol., 46, 255-260, 1958.

Remington, Jack S.; Jacobs, Leon,; Melton, Marjorie L.; and Kaufman, Herbert E.: Chronic Toxoplasma infection in a human uterus. J. Parasitol. 44, 593, 1958.

Honors and Awards relating to this Project: None

Serial No. NIAID-127-B
1. Tropical Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on amebiasis

Principal Investigator: Harry D. Baernstein

Other Investigators: Lucy V. Reardon, Leon Jacobs

Cooperating Units: None

Man Years (calendar year 1958):

Total:	1 1/3
Professional:	2/3
Other:	2/3

Project Description:

Objectives:

The long-range objectives of investigations in amebiasis are concerned with the mechanisms of pathogenesis of Entamoeba histolytica, its metabolsim, methods of diagnosis, factors of host susceptibility and strain virulence, and chemotherapy. The cultivation of the ameba without associated organisms is fundamental to the prosecution of these studies, although attempts can be made toward solving certain problems with the use of present cultures containing single bacterial species.

Methods employed:

The methods include the development, maintenance, and use of cultures of E. histolytica with a number of species of bacteria, for the production of cysts in vitro; cultures with Trypanosoma cruzi as a substitute for bacteria; and cultures with cell-free homogenates of chick embryonic tissues; employment of biochemical techniques adapted to the problem; the use of a micro-isolation apparatus and of microcultures.

Major Findings:

While growth of Entamoeba histolytica has been accomplished in microcultures without associated organisms on a limited scale, continued attempts at defining growth factors in the sub-cellular particles which are essential to such growth, have proved unsuccessful. Uncontrollable variation occurred in microcultures and detracted from the accuracy of growth factor studies by this technique.

The amebiasis project has concerned itself primarily with the production of cysts in culture, for use in microisolation to establish cultures of the ameba with Trypanosoma cruzi. These cultures have been supplied to the Section on Germ-free Studies for experimental use in the production of amebic infections in germ-free guinea pigs.

The only other project carried out has been a study of the usefulness of cultures with glucose rather than starch for the production of antigens. Such cultures may avoid adsorption of antigenic materials on the starch particles present in the usual medium.

Significance to the Program of the Institute:

Definition of growth factors of Entamoeba histolytica should contribute information of use in relation to other micro-organisms and to cells. There are striking similarities between the requirements of this parasite and of helminths that have been cultivated in vitro, and also with the requirements of mammalian cells in vitro. Thus, the prosecution of this program may lead to important basic information applicable to a wide variety of problems.

There is a dearth of information on the pathogenesis of E. histolytica, such as on the mechanisms involved in cytolysis and invasion of tissues, on criteria for distinguishing pathogenic and nonpathogenic strains, and on the significance of the surprisingly low degree of cellular reaction that is attendant on invasive amebae. The answers to these questions lie in the physiology and biochemistry of E. histolytica, which is in turn dependent on pure cultures. Knowledge of immune phenomena is similarly dependent on such cultures.

Proposed Course of the Project:

More active prosecution of studies on amebiasis will be resumed in January, 1959, when a new employee will be assigned to the project.

The immediate aims will be: (1) The cultivation of E. histolytica in a variety of media, with a variety of associates, for the purpose of producing antigens suitable for use in a hemagglutination test for amebiasis or for the exploration of other immunological means of diagnosis, such as passive cutaneous anaphylasis; (2) The development of tests for antibodies in intestinal mucus; (3) The description of hypersensitivity phenomena related to the production of liver abcess or amebic hepatitis in experimental animals; (4) The elucidation of factors involved in encystation and excystation of amebas; and (5) The evaluation of factors associated with the pathogenicity of strains of the parasite. The long-range aim of the program will continue to be the axenic cultivation of E. histolytica in a defined medium, which would permit extensive work on the metabolism of the parasite and would facilitate study of some of the problems mentioned above.

Part B included - No

Serial No. NIAID-127-C
1. Tropical Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Genital trichomoniasis

Principal Investigator: Harry D. Baernstein

Other Investigators: Lucy V. Reardon, Leon Jacobs

Cooperating Units: Laboratory of Pathology and Histochemistry, NIAMD

Man Years (calendar year 1958):

Total:	3 1/3
Professional:	2
Other:	1 1/3

Project Description:

Objectives:

The study of (1) the enzymes of Trichomonas vaginalis in order to provide a biochemical explanation for physiological findings, to improve methods of cultivation, and to provide a biochemical basis for pathogenesis and for chemotherapeutic work; (2) the pathogenesis of T. vaginalis; (3) criteria for differences in strains of trichomonads; (4) antibody-formation and hypersensitivity in relation to resistance and pathogenesis; and (5) the action of purported trichomonicidal agents in experimental infections.

Methods Employed:

Trichomonas vaginalis, grown in large quantities, is ruptured by various means, and the mixture of liberated enzymes is then subjected to fractionation and assay of specific activities which are also related to protein concentration in the samples.

Pathogenesis of strains of Trichomonas vaginalis is tested in various strains of mice and other animals, to find criteria for evaluating strain differences and to relate the differences in strains to the pathology observed in their original human host. Antigens prepared from cultures by various means are tested for their usefulness, especially in the hemagglutination test, with the aim of studying

not only diagnosis, but also hypersensitivity factors involved in pathogenesis.

Major Findings:

Three enzymes linked to diphosphopyridine nucleotide (DPN) have been found and partially characterized. These are: lactic dehydrogenase, malic dehydrogenase, and DPNH oxidase. A 10-fold purification of lactic dehydrogenase was achieved. Preparation of acetone powders of the parasites before sonication affected these enzymes in different ways: The oxidase was completely inactivated probably owing to the loss of its prosthetic group; about 75% of the lactic dehydrogenase was lost; malic dehydrogenase was unaffected. This differential effect may be due in part to differences in distribution of the enzymes in the cell since delaying the acetone treatment until after the cells are ruptured abolished the differences.

The infectivity and pathogenicity of Trichomonas vaginalis was studied in mice. One strain, "R", well established in culture, was used to initiate the work. Standardized inocula of concentrated harvests injected intraperitoneally into male weanlings of NIH General Purpose mice produced minimal, transient, lesions in the form of small caseous masses. Inbred mice of the A/LN strain were then used for intraperitoneal and sub cutis injections; minimal lesions were produced with a second strain of T. vaginalis, "C-1," isolated from a case of severe vaginitis, larger subcutaneous abscesses developed and of the mice injected intraperitoneally, 100 per cent were dead by the 7th day. Concomitant agents were ruled out by appropriate tests.

Susceptibility of 4 additional inbred strains of mice to the R and C-1 parasites was tested. None of the mice was susceptible to the R strain parasite. With C-1 little difference was noted from the A/LN mice except possibly for the C-57 black mouse.

In moribund mice (C-1 strain) the usual pathology consisted of prurulent matter in the peritoneal cavity with large caseous masses on the stomach or intestines; in addition large areas of necrosis were present in the liver. Active trichomonads were present throughout. In collaboration with Dr. Ashburn of the Laboratory of Pathology and Histochemistry of NIAMD, the histopathology was studied. Invasion of the liver proceeds in a wave-like manner leaving behind necrotic liver and always showing a band of parasites at the forward edge of the process.

The pronounced difference between the R and C-1 trichomonads prompted the testing of 11 additional strains. Among these strains there was a wide range of virulence.

In collaboration with the Section on Germ-Free Animal Studies, experiments were set up to assess the results of inoculating germ-free guinea pigs with the R and C-1 strains of Trichomonas. Subcutaneously, C-1 produced lesions of extensive area, usually consisting of large palpable abscesses frequently distended with gas. In comparison the R strain lesions were small. Intraperitoneally, the injections showed little effect.

The effect of the virulent C-1 strain of T. vaginalis in mice has been used as a tool for systemic chemotherapeutic tests with purported trichomonicides. Efforts were made to develop a serological test for T. vaginalis. Antigens were prepared from distilled water lysates of the organism and guinea pigs were immunized by intramuscular injection of these antigens as well as by intraperitoneal inoculation of living concentrates of T. vaginalis. The results, using a hemagglutination test, appear promising.

Significance to the Program of the Institute:

The elucidation of the biochemical properties of Trichomonas vaginalis is one of the basic requirements to an understanding of the relations existing between parasite and host and of the modes of pathogenesis of the parasite in various sites in the body. For example, the pathology produced in the liver of experimentally infected mice may be due to metabolic competition of the trichomonads. The apparent preference of T. vaginalis for polysaccharides such as glycogen may be of significance here and also in relation to vaginal infection. The accumulation of biochemical data is also expected to lay the groundwork for clarification of the relation of the parasite to its associates in the vaginal tract, and the preparation of purified protein fractions of parasitic organisms can be useful in immunologic studies.

Trichomoniasis is a very prevalent infection, responsible for symptoms of varying severity in nearly every case. Investigations on this parasite and its associated conditions may enable the Institute to contribute to a decrease in morbidity and in the physical and nervous discomfort associated with the parasite's presence. The demonstration of the characteristics of the organism related to its virulence is of considerable basic importance, and the fact that some strains of T. vaginalis can produce fatal disease in mice is an experimental argument that the protozoan itself is pathogenic. The infections in mice can be used for studying systemic chemotherapeutic agents, in contrast to topical agents which are rarely completely successful. The encouraging results on the hemagglutination test indicate approaches to studies of antibodies in vaginal mucus as well as serum.

Proposed Course of Project:

The work on this project necessarily has been shifted from a primarily clinical to an experimental approach. This has occurred not solely because of personnel changes, but because the need exists for more laboratory tools and data to support a clinical program.

It is proposed to continue the study of extracts of T. vaginalis for other specific enzymes of the tricarboxylic acid cycle and especially those responsible for CO_2 - and H_2 - production. The excretion of considerable amounts of succinic acid is another phase of metabolism which needs clarification. A study is also proposed to attempt to relate qualitative and quantitative differences in gas-production to the virulence of trichomonad strains.

Work will continue on the pathogenesis of T. vaginalis strains for mice and other animals, and on the pathology of the lesions produced. Alterations in glycogen stores in the liver, prior to infection of mice, will be studied for their effects on the pathology produced in experimental trichomoniasis. The hemagglutination test will be studied further in animals, and possibly in human serum and vaginal exudate.

Part B included - Yes

Serial No. NIAID-127-C

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burch, Thomas A., Rees, Charles W., and Kayhoe, Donald E. Laboratory and clinical studies on vaginal trichomoniasis. J. Obstet. and Gyn. 76: 658-665, 1958.

Burch, Thomas A., Rees, Charles W., and Reardon, Lucy V. Diagnosis of Trichomonas vaginalis vaginitis. J. Obstet. and Gyn. (in press).

Burch, Thomas A., Rees, Charles W., and Reardon, Lucy V. Epidemiological studies on human trichomoniasis. Am. J. Trop. Med. Hyg. (in press).

Burch, Thomas A., Rees, Charles W., and Reardon, Lucy V. Vaginal trichomoniasis and associated cytology. Proc. I. Pan American Cancer Cytology Cong. (in press).

Honors and Awards relating to this project: None

Serial No. NIAID-128-A
1. Tropical Diseases
2. Tropical Arthropod-borne Viral Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on tropical arthropod-borne viruses in tropical and subtropical areas of the United States and Middle America

Principal Investigator: William L. Pond

Other Investigators: Robert M. Pennington

Cooperating Units: Pan American Sanitary Bureau
Ministry of Health, Guatemala
University of Miami School of Medicine
Southwest Blood Banks, Inc.
Gorgas Memorial Laboratory, Republic of Panama

Man Years (calendar year 1958):

Total:	4
Professional:	2
Other:	2

Project Description:

Objectives:

To determine which tropical arthropod-borne viruses are present and active in Central America and in the Southern United States and to assess their relative importance as a cause of disease in these areas.

Methods Employed:

Carry out neutralization, hemagglutination inhibition, and complement fixation tests for arthropod-borne viruses on sera obtained by other investigators from residents of Central America and the Southern United States. Attempt to isolate infectious agents from specimens obtained in Guatemala from patients with febril infections possibly caused by arthropod-borne viruses, also from indigenous mammals, and arthropod vectors.

Major Findings:

Studies of the sera from human beings in selected localities in Guatemala have indicated present or past activity of the viruses of St. Louis encephalitis, Ilheus, Western equine encephalomyelitis, Eastern equine encephalomyelitis, and Yellow Fever. The populations of the lowland areas were especially involved whereas the residents of the mountainous areas were less effected. Studies of residents of the Republic of Panama yielded similar results. Tests on sera from Miami residents indicate the activity of St. Louis encephalitis, encephalomyocarditis, and possibly even Ilheus virus.

*Characterization of viruses isolated by members of the Gorgas Memorial Laboratory and by the Middle America Research Unit are underway.

Significance to the Program of the Institute:

The potential and actual importance of the tropical arthropod-borne viruses as a cause of infection of residents of Central America and the Southern United States should be determined. This knowledge is a prerequisite to the study of tropical arthropod-borne viral diseases.

Proposed Course of the Project:

Sera, collected from residents of Guatemala; Republic of Panama; the Canal Zone; Miami, Florida; El Paso, San Antonio, Harlingen, and Houston in Texas are now available in the Section on Tropical Arthropod-borne Viral Diseases for testing. It is intended that neutralization, hemagglutination inhibition, complement fixation tests will be carried out on the sera not already tested. It is expected that the serum collections now on hand will be augmented with additional sera for testing from the same areas as well as areas in nearby countries.

Part B included - No

Serial No. NIAID-128-B
1. Tropical Diseases
2. Tropical Arthropod-borne Viral Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of arthropod-borne viruses in tissue culture

Principal Investigator: Charles R. Rosenberger

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To study the cytopathogenicity and growth characteristics of the arthropod-borne viruses when cultured in all available tissue culture cell lines. It is hoped that the most satisfactory tissue culture lines of all those now available can be selected for the essential tasks of isolating and characterizing arthropod-borne viruses and for making antigens.

Methods Employed:

Each arthropod-borne virus, in order of importance, is being tested in all tissue culture lines for evidence of cytopathogenicity and viral growth. Visual observations are made daily for evidence of cytopathogenicity and the materials are inoculated into mice or into other tissue culture lines for determinations of the amount of viral growth.

Major Findings:

Of 15 different arthropod-borne viruses and 13 different tissue culture cell lines studied, the hamster kidney cell line appears to offer the most promise as a cell line satisfactory for supporting viral growth and indicating its cytopathogenicity. The dengue viruses are still outstanding in their lack of consistent pathogenicity in any tissue culture line.

Significance to the Program of the Institute:

The proper evaluation and selection of just a few satisfactory tissue culture cell lines for use in primary isolation and characterization of viruses from clinical and other materials will be of great value not only to the activities of the Section on Tropical Arthropod-borne Viral Diseases but also to the Middle America Research Unit in Panama and to all other laboratories engaged in studies of arthropod-borne viral diseases.

Proposed Course of the Project:

It is expected that the program briefly described above will be continued and expanded to cover most of the important arthropod-borne viruses now numbering 78 in all. Considering the effort required to carry out a satisfactory evaluation of this type, a program lasting one to two years will be required.

Part B included - No

Serial No. NIAID-128-C
1. Tropical Diseases
2. Tropical Arthropod-borne Viral Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Effects of artificially varied environment on potential mosquito vectors of arthropod-borne viruses

Principal Investigator: Paul A. Woke

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To determine the optimum conditions for the maximum survival of potential mosquito vectors of arthropod-borne viruses and to learn the effects of controlled suboptimal conditions on the same mosquito species. To colonize species of mosquitoes suspected as being of importance in the dissemination of arthropod-borne viruses.

Methods Employed:

Various mosquito species, considered important as vectors of arthropod-borne viruses, have been observed at conditions of temperature and humidity considered optimal for survival for that species as well as at extreme conditions in order to determine their tolerance. The criteria used have in general been expressed in terms of the survival of the mosquitoes under the conditions used and survival during subsequent observation.

Major Findings:

Adult Haemagogus equinus females have survived for a maximum of 69 days, males for 64 days, and mating occurs, under laboratory conditions of

25°C, 65-70% RH, and 14 hours of artificial white light per day varied in intensity from under one foot candle to 58 foot candles. Mortality rate is increased at temperatures of 20° and 30°. A part of the population can survive temperatures of 5°C for 24 hours.

H. mesodentatus adults perform all normal activities in small laboratory cages except mating. Copulation with successful insemination can be induced by unnatural means. Haemagogus mosquitoes can be confined and fluids can be presented to masses of mosquitoes by newly developed means which remove any likely chance of injury or entrapment which occurred with previously used methods.

Significance to the Program of the Institute:

Maximum survival rate and longevity of mosquito vectors under optimal and suboptimal laboratory conditions indicates healthy experimental stock and provides necessary conditions for biological and virus-transmission experimentation. The ability to survive low temperatures is indicative of survival and geographical distribution potentials under natural conditions and provides information of value in arranging transportation from natural habitats. The possibility of inducing copulation in a vector species which has not been colonized provides a needed means by which to propagate the species in the laboratory for limited experimental use.

Removing the likelihood of injuries under caged conditions saves much time and effort, makes possible certain biological determinations that otherwise would be impossible, and aids in all laboratory experimental research. Knowledge gained from a study of the ecology and behavior of the vectors is of the greatest importance as a basis for further studies in transmission and for the development of control measures.

Proposed Course of the Project:

Through studies of expected vectors of viruses in the laboratory and in the natural habitat, to improve our understanding of the environmental conditions that are necessary for maximum survival and longevity, mating, and egg-production in the laboratory, of feeding behavior in the laboratory and in the habitat, and of environment in relation to virus transmission.

Part B included - No.

Serial No. NIAID-128-D
1. Tropical Diseases
2. Tropical Arthropod-borne Viral Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Transmission of arthropod-borne viruses by mosquitoes

Principal Investigator: Herbert E. Delmat

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To determine the interrelationships of mosquitoes and the tropical arthropod-borne viruses. To develop strains of viruses in appropriate laboratory animals that will be infective for mosquitoes and to determine the viremia pattern in them. To determine extrinsic incubation periods, susceptible mosquitoes, site of multiplication in the mosquito, and optimal methods for demonstrating viruses in the mosquitoes.

Methods Employed:

Infect animals with various strains of virus, titrate their blood in mice to determine viremia levels, feed mosquitoes on infected animals at the peak of viremia, inoculate suspensions of infected mosquitoes or of pools of their body parts into susceptible hosts to determine the presence and the titer of virus, refeed infected mosquitoes on susceptible hosts at appropriate intervals after their infective feeding to determine the extrinsic incubation periods and ability of the species to transmit.

Major Findings:

Transmission studies have been carried out with Ilheus virus and the mosquitoes Culex quinquefasciatus and Aedes aegypti. It was found that mouse-adapted strains of Ilheus virus produced viremia in mice and chicks which was too brief and too low to infect either of the species of mosquitoes used. It will be necessary to produce a chick-adapted strain or secure wild strains of virus in order to pursue the studies of mosquito-virus relationships further.

Significance to the Program of the Institute:

With our present knowledge of the presence of certain arthropod-borne virus diseases in the United States and of the possible introduction of others from countries to our south, we must learn more about the role of the vector species: Which arthropod species actually transmit the diseases? Do they play a role in maintaining the virus during the winter months or when the disease is not patent in humans or domestic animal hosts? What mammals may serve as reservoir hosts and which arthropods transmit from them to humans and domestic animal hosts? This program will attempt to help answer some of these questions.

Proposed course of the Project:

Continue with objectives and methods already stated.

Part B included - No

NIAID
Laboratory of
Immunology

Estimated Obligations for FY 1959

Total:	$368,100
Direct:	285,000
Reimbursement:	83,100

Covering Project Reports
NIAID....140 thru 154
Project 149 canceled before start of
FY 1959

Form No. ORP-2
Nov. 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NIAID - 140
1. Laboratory of Immunology
2. Basic Immunology
3. Bethesda, Maryland

Part A

Project Title: Studies in the Transplantation of Tissues

Principal Investigator: Dr. Joseph A. Bauer, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1.0
Professional: .50
Other: .50

Patient Days: None

Project Description:

Immune mechanism of homologous skin graft rejection.

Objectives:

a. To explore the similarities between the tuberculin type of delayed hypersensitivity and the immunity resulting in response to foreign tissue grafts.

b. To transfer homograft immunity passively by the use of cells and/or serum in inbred strains of guinea pigs.

c. To develop a skin test for the measurement of homotransplantation immunity by injecting cells intradermally.

d. To investigate the genetic laws of tissue transplantation by various breeding combinations of inbred guinea pig strains.

Methods Employed:

a. Skin grafts are used as the model system for the evaluation of the fate of tissue transplants. A standardized reproducible technique using a dermatome was developed for the preparation of split-thickness skin grafts.

b. Lymphoid cells or serum from inbred guinea pigs immunized to homografts by various means are transferred passively to normal guinea pigs of the same strain bearing test skin grafts from another strain.

Major Findings:

a. Two inbred strains of guinea pigs were found to be genetically identical to the extent of allowing permanent survival of skin grafts exchanged between individual animals within each strain. Skin grafts exchanged between animals belonging to different strains show a characteristic slough reaction.

b. A second skin graft applied following (1 - 20 weeks) the rejection of a graft of the same donor strain shows an accelerated rejection characterized by the "white graft" reaction.

c. The acceptance of grafts from both parent strains by F_1 hybrid guinea pigs (but not the reverse), as well as the histocompatibility among F_1 hybrids, further strengthens the prior finding of a high degree of inbreeding in the two strains of guinea pigs studied.

d. Skin testing with living lymphoid cells results in skin reactions resembling in appearance and time course the delayed tuberculin type hypersensitivity reaction.

e. Passive transfer of homograft immunity with large volumes of "immune" serum have failed; passive transfer with living immune lymphoid cells were often successful. Both findings are in agreement with the "cell fixed" noncirculating nature of homograft immunity.

Significance to the Program of the Institute:

It is essential that highly inbred histo-compatible animals be used for studies of tissue transplantation. Previous to the above findings, only mice, and to a lesser degree rats, were available for transplantation work. Guinea pigs, aside from being the largest animals with demonstrated histo-compatibility, offer several other advantages particularly in studying the delayed "cell fixed" nature of homograft immunity.

Proposed Course of Project:

a. Experiments in progress attempting to alter the destructive response to foreign tissue transplants in guinea pigs by (a) induction of immunological tolerance to foreign tissues and by (b) reduction of serum complement in recipient animals will be continued.

b. Cell transfers to "tolerant" guinea pigs will be carried out in an effort to elucidate the nature of tolerance to foreign grafts.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 140

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications:

Bauer, Joseph A.: Histocompatibility in Inbred Strains of Guinea Pigs. Ann. New York Acad. Med. 73:663-672, 1958.

Form No. ORP-2
Nov. 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NIAID - 141
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

PART A

Project Title: Studies on Antibody Production and Hypersensitivity

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: Dr. Jules Freund

Cooperating Units: None

Man Years:
Total: 2.33
Professional: 1.33
Other: 1

Patient Days: None

Project Description:

Objectives:

To study the immunology, physiology and pathology of protracted anaphylactic shock.

Methods Employed:

Guinea pigs are sensitized actively by injecting egg albumin with or without paraffin oil adjuvants. The mechanism of anaphylactic shock is being studied and the role of circulating antibody in anaphylaxis determined using measured amounts of guinea pig antibody to sensitize normal guinea pigs. Protracted anaphylactic shock caused by injection in the subcutaneous tissue is the experimental model.

Major Findings:

Prolongation of anaphylactic shock in guinea pigs is a function of circulating antibody. Antigen combining with antibody present in the lungs is the cause of swift death from bronchospasm in acute anaphylactic shock, but circulating antibody will retard the absorption of antigen from a subcutaneous depot, preventing bronchospasm. The guinea pigs may die after several hours in protracted shock. Death is not due to bronchospasm.

Guinea pigs in protracted shock show several striking physiological changes. During protracted anaphylaxis, Arthus reactions are inhibited. In guinea pigs immunized with egg albumin in paraffin oil adjuvants containing mycobacteria, but not in those immunized without mycobacteria, large hemorrhagic reactions occur at the site of subcutaneous injection of large amounts of egg albumin.

Significance to the Program of the Institute:

Protracted anaphylactic shock in the guinea pig has been known as a rare occurrence and was not studied. The conditions necessary for its production were not well established. Protracted anaphylaxis in man is quite important because it occurs after the subcutaneous or intramuscular injection of allergenic material. Its incidence is higher than that of acute anaphylactic shock with predominantly respiratory symptoms.

Some clarification of the mechanism of anaphylactic shock is possible, and some unification of current theories of this mechanism is achieved.

Studies on the biological activity of tubercle bacilli in hypersensitivity permit some comparison of delayed hypersensitivity components (cellular "antibodies") and immediate hypersensitivity components (circulating antibodies) in "allergic" shock of a protracted nature.

Proposed Course of Project:

The role of circulating antibody and antigen-antibody complexes in anaphylactic shock will be further studied.

Part B included Yes /X/ No /_/

(Attachment 1)

Serial No. NIAID - 14

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

Stone, S. H.: Protracted anaphylactic shock after subcutaneous eliciting injection. Fed. Proc. 17:536, 1958.

Stone, S. H.: Inhibition of Arthus reaction during protracted systemic anaphylaxis in mice and guinea pigs. Proc. Soc. Exper Biol. and Med. 98:269, 1958.

Stone, S. H.: Further studies on protracted anaphylactic shock in guinea pigs after subcutaneous eliciting injection. Excerpta Medica, III rd. International Congress of Allergology, October, 1958, p. C70.

Stone, S. H.: Anaphylaxis in passively sensitized guinea pigs after subcutaneous eliciting injection. Science, 128:1090, October 31, 1958.

Stone, S. H. and Freund, J.: Hemorrhagic and necrotic reactions in guinea pigs during protracted anaphylactic shock. Proc. Soc. Exp. Biol. Med. 99:333, 1958.

Stone, S. H.: Acute and protracted anaphylactic shock in guinea pigs after subcutaneous eliciting injection of antigen. J. Immunol. In Press.

PART A

Project Title: The Application of the Bentonite Flocculation Test as a Laboratory Aid for the Diagnosis of Certain Diseases and the Application of the Bentonite Flocculation Test as a Tool for Immunologic Studies

Principal Investigator: John Bozicevich

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 3
Professional: 1
Other: 2

Patient Days: None

Project Description:

Objectives:

1. To coat bentonite particles with poliovirus to determine whether flocculation will occur with sera obtained from (a) rabbits injected with poliovirus, (b) patients at certain stages of polio virus infection, (c) individuals immunized with poliovirus vaccine.

2. To coat bentonite particles with certain allergens, for example cottonseed and ragweed and to determine whether flocculation will occur in sera obtained from (a) rabbits injected with respective antigens, (b) indivisuals naturally sensitized, (c) normal volunteer individuals following injections of varying amounts of the allergens.

3. The Bentonite Flocculation Test is being studied in relation to the quantitative precipitin (antibody nitrogen determination) test

Methods Employed:

Poliovirus vaccine is dialyzed for several days against running tap water. The material is lyophilized and extracted with various salts. These extracts are used to sensitize the bentonite particles. The specific sensitized bentonite particles are tested against sera obtained as outlined under Objectives, paragraph 1.

Bentonite particles are coated with ragweed and cottonseed allergens. Large particle size allergens (those remaining within the dialyzing membrane), and small particle size (those passing through the dialyzing membrane) are used to sensitize the bentonite particles. Nitrogen determinations on both products are conducted and tested as outlined under Objectives, paragraph 2.

In conducting the quantitative precipitin tests, bentonite particles sensitized with the specific antigen under investigation are used on the supernates to test for excess antigen and antibody.

Major Findings:

Bentonite particles sensitized with poliovirus vaccine gave specific flocculation tests with sera obtained from rabbits which were injected with live poliovirus. In several instances the titer was greater than that obtained by complement fixation tests. In two instances positive results were obtained on the sera of individuals with poliovirus infection. A rise in titer was obtained in serum samples after CNS symptoms. In individuals who had received an immunization course with poliovirus vaccine, the bentonite flocculation test gave negative results.

The sera of rabbits immunized with ragweed antigen gave positive bentonite flocculation tests when tested with bentonite particles coated with ragweed allergens. Negative results were obtained on sera from individuals sensitive to ragweed. Furthermore, the flocculation tests were negative in sensitive individuals who had received a desensitizing course.

The bentonite flocculation tests were positive with sera from rabbits which had been immunized with cottonseed allergen. Positive tests were given with bentonite particles sensitized with a large molecule (particle size not passing through dialyzing membrane, 17.1%N), and also with small size molecules (passing through dialyzing membrane, 13.3%N).

Last year we reported that the Bentonite Flocculation Test could detect (0.04 to 0.06 micrograms antibody nitrogen) 80 to 100 times smaller amounts of antibody than the precipitin test. The sensitivity of the test has now been increased to detect as little as 0.01 to 0.03 micrograms of antibody nitrogen.

Significance to the Program of the Institute:

A sensitive poliovirus test may replace the expensive Metabolic Inhibition Test for poliovirus infection detection. A sensitive test which would indicate antibody titer may be useful following poliovirus vaccination.

Proposed Course of the Project:

To continue studies to improve the sensitivity and specificity of the Bentonite Flocculation Test for various diseases.

Part B included Yes [X] No [ ]

(Attachment 1)

Serial No. NIAID - 142

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

Bozicevich, John, Bunim, Joseph J., Freund, Jules and Ward, Stanley B.: Bentonite Flocculation Test for Rheumatoid Arthritis. Proc. Soc. Exp. Biol. and Med., 97:180, 1958.

Bozicevich, John: The Serology of Amebiasis. 6th International Congresses of Tropical Medicine and Malaria, Lisbon, Portugal, 1958, In Press.

Form No. ORP-2
Nov. 1958

Serial No. NIAID - 143
1. Laboratory of Immunology
2. Basic Immunology
3. New York, New York

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Characterization of Substances of Bacterial Origin Affecting Resistance to Infection

Principal Investigator: Dr. Curtis A. Williams, Jr.

Other Investigators: None

Cooperating Units: Dr. Rene J. Dubos, Rockefeller Institute; Dr. Jerome W. Schulman, Department of Preventive Medicine, Cornell University Medical School

Man Years:

Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description:

Objectives:

1. Further examination of biologically active materials extracted from tubercle bacilli. Their isolation and composition.

2. To distinguish between the mechanisms of specific and non-specific acquired resistance to infectious diseases.

Methods Employed:

1. Animal protection tests are performed in albino mice. They are studied by bacterial enumeration from infected organs and by survival time.

2. Studies of hypersensitivity and adjuvant activity are carried out in albino guinea pigs.

3. Cockroaches, having slight if any humoral defense system, are being adapted to the investigation of cellular defenses against infection with bacteria.

4. Biologically active substances from tubercle bacilli are compared with bacterial endotoxins from Gram negative organisms, which have similar chemical composition and most of the same activities.

Major Findings:

1. A fraction of a methanolic extract of tubercle bacilli capable of inducing a specific resistance to tuberculosis is water soluble and active in fairly small amounts. So far it has not been purified.

2. A substance insoluble in 33% ethanol and 0.5% sodium chloride behaves in several respects like endotoxins of Gram negative bacilli. Properdin is complexed, mice are rendered non-specifically resistant to bacterial infections, and the nitrogen, phosphorus and carbohydrate contents are similar to those of the Gram negative lipopolysaccharides.

3. All materials from tubercle bacilli tested so far have proven useless as adjuvant, although the so-called "Wax D" of other investigators (also presumed to be lipopolysaccharide) is reported to have this activity.

4. Preliminary results with cockroaches indicate that infections can be modified in this host by the administration of Gram negative endotoxins much the same way as in mice.

Significance to the Program of the Institute:

The study of the chemical and immunological nature of fractions from tubercle bacilli is very significant from the standpoint of the production of a prophylactic vaccine against tuberculosis. Information on the fractions of tubercle bacilli may give insight into the pathogenesis and immunity of tuberculosis.

Proposed Course of Project:

Further experiments designed to elucidate the nature of specific and non-specific resistance as demonstrated by the administration of bacterial products.

Part B included Yes [ ] No [X]

Part A

Project Title: Pathogenesis of Experimental "Allergic" Encephalomyelitis

Principal Investigator: Dr. Philip Y. Paterson

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description:

Study of the pathogenesis of experimental "allergic" encephalomyelitis is being conducted under contractual arrangement between the National Institute of Allergy and Infectious Diseases and the New York University College of Medicine (Contract Serial No. SA-43-ph-1808).

Experimental "allergic" encephalomyelitis (EAE) is induced in adult albino rats by an injection of brain or spinal cord emulsified in complete adjuvant (mycobacteria, paraffin oil, and emulsifying agent).

1. Project Subtitle: Passive Transfer of Allergic Encephalomyelitis

Objectives:

Transfer of allergic encephalomyelitis to rats by means of cells from lymph nodes of actively sensitized rats.

Methods Employed:

Prospect recipient rats are either (a) injected intraperitoneally 1 to 12 days after birth with pooled normal splenic cells from groups of adult splenectomized rats or (b) not injected with splenic cells during neonatal life. When 5 to 6 weeks old, these rats are used as recipients in cell transfers and are injected intravenously with washed immune lymph node cells from donor rats previously injected intracutaneously with guinea pig spinal cord emulsified in complete adjuvant. Recipient rats are observed for clinical neurological signs for 6 to 8 days following cell transfer, then sacrificed and their brains and spinal cord removed for histological

examination. "Immune" lymph cells for transfer are obtained from pooled cervical-axillary lymph nodes collected from donor rats usually 7 days (occasionally 12 days) after injection of spinal cord-adjuvant. Suspensions of "immune" lymph cells transferred are from either (a) the groups of previously splenectomized donor rats or (b) other groups of non-splenectomized donor rats. Using these 2 types of recipient animals and these 2 types of donor groups, the following types of cell transfers are made. In type A transfers, "immune" lymph cells from splenectomized donor rats are transferred into recipients injected neonatally with normal splenic cells from these same rats. In type B and type C transfers, "immune" lymph cells from non-splenectomized donor rats are transferred into recipients injected neonatally with normal splenic cells from different rats and into recipients not injected neonatally with splenic cells, respectively. The donor/recipient ratios in the various cell transfers vary from 1/2 to 4/1. Individual recipient rats receive from 10 to 110 million viable nucleated immune lymph cells as determined by vital staining and cell counts of the cell suspensions transferred.

Major Findings:

Type A cell transfers (splenectomized donor rat "immune" lymph cells into recipients injected neonatally with normal spleen cells from these same donors) regularly results in transfer of allergic encephalomyelitis. More than half (63% to date) of the recipients show typical lesions of allergic encephalomyelitis. The severity and extent of the lesions in individual recipient animals can be directly correlated with the number of "immune" cells transferred. Mild clinical neurological signs (ataxia, hind leg weakness) appear in a few recipients showing intense and widely disseminated focal lesions in the central nervous system.

Type B cell transfers (non-splenectomized donor rat "immune" lymph cells into recipients injected neonatally with normal spleen cells from different rats) occasionally result in transfer of allergic encephalomyelitis. Less than half (27% to date) of the recipients show lesions of the disease. Successful transfers in these instances appear related to the use of large numbers of transferred immune cells (more than 70 million cells per recipient), a high donor/recipient ratio, and relatively late collection of cells from donor animals (12 days after injection of spinal cord-adjuvant).

Type C cell transfers (non-splenectomized donor rat "immune" lymph cells into recipients not injected neonatally with normal spleen cells) does not result in transfer of allergic encephalomyelitis under the conditions as described. This finding agrees with the results of many cell transfers of this type made previously in our laboratory.

The presence of typical lesions in recipient rats 6 to 8 days after cell transfer and the absence of lesions in the actively sensitized donor rats 7 days after injection of spinal cord-adjuvant indicates that the disease in the recipient animals is not due to active sensitization. The lack of significant tissue damage in other organs (liver, spleen, kidneys, and lymph nodes) collected from some of the recipient rats indicates the organ-specificity of the disease transferred. The successful passive transfers may be due, at least in part, to a favorable environment in the recipient rats. These recipients were injected neonatally with normal splenic cells which is known to induce homograft tolerance; prolonged survival of transferred donor "immune" cells can be assumed in these recipients and may be important for transfer of this disease.

Significance to the Program of the Institute:

Successful passive transfer of allergic encephalomyelitis represents long awaited evidence that this disease has, indeed, an immunological mechanism. This work provides a means for answering several important questions about the nature of allergic reactions to tissue antigens.

Proposed Course of Project:

This project will be continued in order to more fully define the conditions required for successful passive transfer of allergic encephalomyelitis and determine the importance of cellular and humoral factors in the pathogenesis of this disease.

2. Project Subtitle: Immunological Tolerance to Nervous Tissue

Objectives:

To determine optimal time and minimal amount of nervous tissue required for induction of tolerance to the paralytogenic activity of nervous tissue.

Methods Employed:

Litters of rats are injected with different amounts of whole guinea pig spinal cord homogenized in saline when 1, 9, 14, or 28 days old. Controls consist of littermates similarly injected with different amounts of guinea pig kidney homogenized in saline or left uninjected. All rats in each litter are later injected intracutaneously with guinea pig spinal cord emulsified in complete adjuvant when 8 to 10 weeks old, the animals are subsequently observed for clinical neurological signs for approximately 3 weeks, then sacrificed and their brains and spinal cords removed for histological examination.

Major Findings:

Injection of 1 day old rats with 16, 10, or 5 mgm of spinal cord per gram body weight induces tolerance to paralytogenic spinal cord-adjuvant emulsions. The majority of these rats show no encephalomyelopathy after injection of spinal cord-adjuvant at 8 - 10 weeks of age. Littermate controls develop typical disease. Injection of 1 day old rats with 1 mgm of spinal cord per gram body weight induces little, if any, tolerance.

Injection of 9 day old rats with 10 mgm spinal cord per gram body weight induces tolerance.

If as much as 16 mgm spinal cord per gram body weight is injected into 14 day old rats, some of these animals later show some evidence of tolerance to paralytogenic spinal cord-adjuvant emulsions. Injection of 10, 5, or 1 mgm spinal cord into 14 day old rats, however, does not induce tolerance.

Injection of 28 day old rats with 5 or 10 mgm spinal cord per gram body weight induces no tolerance.

Significance to the Program of the Institute:

Basic information on immunological tolerance to tissue antigens with particular reference to tolerance to homografts.

Proposed Course of Project:

Project to be continued. Immunological tolerance to homologous nervous tissue and duration of the tolerance to heterologous nervous tissue will be studied.

3. Project Subtitle: Paralytogenic Activity of Neonatal and Adult Rat Brain

Objectives:

To determine when paralytogenic activity appears in rat brain after birth.

Methods Employed:

Rats are sacrificed 1, 9, 14, or 28 days after birth and their brains removed. Brain tissue collected at these different times in complete adjuvant and injected intracutaneously into adult rats and guinea pigs. Occurrence of allergic encephalomyelitis in these adult animals indicates that the brain tissue injected had paralytogenic activity.

Major Findings:

Brains from 1 and 9 day old rats have no demonstrable paralytogenic activity. Slight paralytogenic activity can be demonstrated in 14 day old rat brains. Brains from 28 day old rats are highly paralytogenic. It is important to note that rat brain antigen(s) responsible for induction of allergic encephalitis is first demonstrable at a time, 14 days after birth, when most rats can no longer acquire immunological tolerance to the paralytogenic activity of nervous tissue.

Significance to the Program of the Institute:

Basic information on antigenicity of immature tissues with special reference to use of embryonic tissues for homografting.

Proposed Course of Project:

Project concluded.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 144

PHS-NIH
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Part B: Honors, Awards, and Publications

Publications:

Paterson, P. Y.: Studies of Immunological Tolerance to Nervous Tissue in Rats. Ann. New York Acad. Sci. 73:811-817, 1958.

1958

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Laboratory of
2. Office of the Chief
3. Bethesda, Maryland

Part A

Project Title: Vascular Permeability Factors in the Secretion of the Guinea Pig Coagulating Gland

Principal Investigator: Dr. Jules Freund

Other Investigators: None

Cooperating Units: Dr. A. A. Miles, The Lister Institute of Preventive Medicine, London, England

Man Years:

Total: .33
Professional: .33
Other: 0

Patient Days: None

Project Description:

Objectives:

To study a constituent in the fluid of the coagulating gland of the guinea pig which is highly active in increasing the permeability of blood vessels.

Methods Employed:

a. Fractionation by starch electrophoresis.

b. Chemical tests for p-toluenesulphonylarginine methyl esterase and caseinolytic activity; tests for protein and carbohydrate.

c. Test for vascular permeability.

Major Findings:

The active fraction contains p-toluenesulphonylarginine methyl esterase, glycoprotein. This fraction, as permeability factor in the rabbit, is sixteen times more potent than histamine compared weight for weight. On molar basis, the fraction is 9,000 times more potent than histamine.

Significance to the Program of the Institute:

Permeability of the blood vessels is one of the most important aspects of the pathogenesis of allergic diseases.

Proposed Course of the Project:

The mode of action of the permeability factor will be investigated.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 145

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

Freund, J., Miles, A. A., Mill, P. J., and Wilhelm, D. L. Vascular Permeability Factors in the Secretion of the Guinea Pig Coagulating Gland. Nature 182:174-175, 1958.

PART A

Project Title: The Role of Phagocytes in Immunity

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .66
Professional: .33
Other: .33

Patient Days: None

Project Description:

Objectives:

Although the site and nature of antibody formation has been studied for many years, much remains to be elucidated. In particular, the exact role of phagocytes in this process remains obscure. To study this problem, the ability of fixed and free phagocytes to passively transfer immunity will be investigated.

Methods Employed:

Immunized animals will be injected with especially dispersed iron particles which are one micron in size. Once these particles have been ingested by the phagocytes, cells will be collected by liver perfusion and the phagocytes separated from other cells by passing the cell suspension over a magnet. In this manner, pure samples of phagocytes will be collected.

Major Findings:

The transfer of white cells, washed from the liver of highly immunized animals, to normal recipients, resulted in the appearance of antibody in these recipients earlier than can be accounted for by antigen transfer.

Significance to the Program of the Institute:

If phagocytes are active in antibody formation, these studies would yield new means of studying antibody formation on cellular level.

Proposed Course of Project:

The technique for separation of the iron containing, from the non iron containing cells will be improved. Transfer studies will then be made with the separated iron containing phagocytes.

Part B included Yes [X] No [ ]

(Attachment 1)

Serial No NIAID 146

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications

McMaster, Philip R. B.: Auto-Antibodies and Autosensitivity. N. Y. State Journal of Medicine, 58:2980, Sept. 15, 1958

Form No. ORP-2
Nov. 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NIAID - 147
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A

Project Title: Immunochemical Studies of the Components of Serum

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years:

Total: 3
Professional: 1
Other: 2

Patient Days: None

Project Description:

Objectives:

a. To study the chemical nature and biological significance of the antigens in serum which induce isoantibodies.

b. To develop methods for the in vitro demonstration of non-precipitating antibodies found in allergic diseases.

Methods Employed:

a. Immunochemical analysis by diffusion in agar-gel; simple diffusion in tubes, double diffusion in plates, and immuno-electrophoresis.

b. Passive cutaneous anaphylaxis.

c. Cellulose ion-exchange chromatography.

d. Zone electrophoresis, ultracentrifugation.

Major Findings:

1. Investigations have shown that components of sera from individual rabbits are antigenic in certain other rabbits and that isoprecipitins can be produced which react with serum antigens having electrophoretic mobilities corresponding to α, β, and γ-globulins. A study of the reactions of isoprecipitins with 350 normal rabbit sera obtained from several breeds of rabbits showed that the individual rabbits contain one or the other or both of two γ-globulin specificities in their sera but never

lack both of them. Of the 350 rabbits tested, 18 had one type of γ -globulin, 288 had another type, and 44 had both types in their sera.

2. A few experiments with the sera of multitransfused patients and with the sera of patients given several subcutaneous injections of human albumin or human gamma globulin failed to reveal the formation of isoantibodies. Preliminary evidence has been obtained which indicates isoantibody formation to serum components of mice and guinea pigs.

Significance to the Program of the Institute:

1. Differences in serum proteins between individuals of the same species may be of genetic significance similar to that of the blood groups. The serum isoantigens may be implicated in some of the unexplained transfusion reactions in man.

2. Serum isoantigens may be useful in the study of immune tolerance and perhaps serve as a somewhat analogous experimental model for the study of supposed tolerance to Rh antigen.

3. Isoantigens demonstrated in red blood cells and lymphoid cells do not occur in solution although isoantigens present in red cells have been extracted and are available in pure solution. The serum proteins which are endowed with isoantigenic properties are the only ones known thus far which occur in solution.

4. Serum protein isoantigens may be particularly suitable for study of some basic problems in immunology concerning the nature of antigen and antibody sites, antigenicity of antibodies, the antigen-antibody reaction, etc.

Proposed Course of Project:

1. The genetic mechanism for the inheritance of γ -globulin isoantigens in rabbits.

2. Naturally occurring and artificially induced immune tolerance to γ -globulin isoantigens in rabbits.

3. Specificity of rabbit -globulin isoantigens and antigenicity in other species.

4. Chemical characterization of γ -globulin isoantigens in rabbits: Fractionation, purification, quantitative determination, electrophoretic mobility, sedimentation constant, enzymatic breakdown products, etc.

5. Comparison of antibody production in rabbits of different γ-globulin types.

6. Study of α and β-globulin isoantigens in rabbit sera.

7. Isoantigens in other species.

8. In vitro demonstration of nonprecipitating antibodies found in allergic diseases.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 147

PHS-NIH
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Part B: Honors, Awards, and Publications

Publications:

Dray, Sheldon, and Young, G. O.: Differences in the Antigenic Components of Sera of Individual Rabbits as Shown by Induced Isoprecipitins. Jour. Immunol. 81:142-149, 1958.

Dray, Sheldon, and Young, G. O.: Two Antigenically Different -globulins in Rabbits as Revealed by Isoprecipitins. Science, In Press.

Part A

Project Title: Basic Studies on Cellular Localization of Antigens, Antibodies, and Other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy

Principal Investigator: Dr. John E. Tobie

Other Investigators: Dr. Jules Freund

Cooperating Units:

These studies on the specific localization of the tetracyclines in abnormal bone formations are being done in cooperation with Dr. Robert A. Milch and Dr. R. A. Robinson of the Department of Orthopedic Surgery, Johns Hopkins University Hospital.

Man Years: Patient Days: None

Total: 3.33
Professional: 1.33
Other: 2.0

Project Description:

Objectives:

1. To determine the specific sites of deposition of the tetracycline antibiotics in the abnormal bone formations of various tumors.

2. To perform studies on the types of cells involved in antibody formation, using the footpads of neonatal and adult rabbits as the sites of injections of antigens and the regional lymph nodes as the sites of antibody formation.

Methods Employed:

1. Tumors were obtained at surgery and the specimens quick-frozen in petroleum ether chilled to -65° C. Thin fresh-frozen sections of the tumors were cut in a cryostat and the fluorescent tetracyclines localized by fluorescence microscopy.

2. Neonatal rabbits (1 - 2 days old) and adult rabbits are being given a single injection of bovine serum albumen into the footpad. As soon as the animals show any circulating

antibody, as determined by a sensitive hemagglutination test, they are sacrificed, the homolateral popliteal lymph node excised and the antibody producing cells determined by the "sandwich" method of the fluorescent antibody technique.

Major Findings:

1. The microscopic localization of the tetracyclines has been discovered in the bone formations of the following tumors: Osteogenic sarcoma, chondrosarcoma, osteochondroma, reticulum cell sarcoma, and the plasma cell myeloma. In all of these tumors the tetracyclines appear to be deposited in the newly proliferated bone and not in old well-established bone. This is in keeping with the localization in normal bone. By the use of histochemical techniques it was further determined that, apparently, the calcium salts must be layed down in these abnormal bone formations before the tetracyclines can be deposited. The fluorescent tetracyclines were not observed to localize in the tumor cells.

2. The experiments on the lymph node cells first involved in the elaboration of antibody are in preliminary stages. In neonatal rabbits it has been noted that as early as two days after injection with bovine serum albumen the homolateral lymph node was approximately two times as large as the heterolateral node suggesting proliferation of the cellular elements. Ten days after injection, the homolateral lymph node of the adult rabbits was about 4 - 5 times as large as the heterolateral node. The adult rabbits produced antibodies within eight days while the neonatal rabbits have produced no detectable antibodies up to thirty days.

Significance to the Program of the Institute:

1. The precise localization of the tetracyclines in the abnormal bone of malignant tumors would seem to offer therapeutic possibilities.

2. The selective deposition of these antibiotics also provides an excellent means whereby histological evidence of specific biological processes in bone may be obtained.

3. The determination of the specific cells involved in antibody production may provide a basis for a better understanding of immune mechanisms. The influence of age upon antibody formation is an important factor in the capacity of infants to be actively immunized.

Proposed Course of Project:

1. The studies on the localization of the tetracyclines in the abnormal bone of tumors will be brought to a conclusion during the ensuing years.

2. The basic studies on the determination of the cells involved in antibody production and the influence of age upon antibody formation will be continued, utilizing fluorescent antibody techniques.

Part B included Yes [X] No [ ]

Serial No. NIAMD - 148

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of Tetracycline Antibiotics in Bone. Jour. Bone Joint Surg. 40-A: 897-910, 1958.

Tobie, John E.: Certain Technical Aspects of Fluorescence Microscopy and the Coons Fluorescent Antibody Technique. Jour. Histochem. Cytochem. 6:271-277, 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. MIAID - 150
1. Laboratory of Immunology
2. Basic Immunology
3. Bethesda, Maryland

PART A

Project Title: The Effect of Local Adjuvant Depots Upon Subsequent Immunization

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: Dr. Jules Freund

Cooperating Units: None

Man Years:
Total: .66
Professional: .33
Other: 33

Patient Days: None

Project Description:

Objectives:

To determine the effect of adjuvants upon immunization when the adjuvant without antigen is given locally, and the antigen is given intravenously.

Methods Employed:

Incomplete adjuvants will be injected locally; subcutaneously in one group of animals, complete adjuvants locally in another group. Particulate and soluble antigens will be injected intravenously in these groups as well as into normal animals. The antibody titers in these animals will be compared as will be the development of delayed sensitivity.

Major Findings:

There are no significant findings as yet.

Significance to the Program of the Institute:

The results will give information about the mode of action of adjuvants. It may also lead to improved techniques for all types of immunization against infectious diseases.

Proposed Course of Project:

To be continued until the results are definite.

Part B included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NIAID - 151
1. Laboratory of Immunology
2. Basic Immunology
3. Bethesda, Maryland

PART A

Project Title: The Significance of Persistant Antigen

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .66
Professional: .33
Other: .33

Patient Days: None

Project Description:

Objectives:

The significance of persistant antigen is not known. This study is designed to determine whether persisting antigen does or does not serve to maintain sensitization. If the persistant antigen serves to maintain sensitization, this study will yield a new approach to desensitization.

Methods Employed:

An artificial antigen will be constructed with a small well defined chemical haptene coupled to a large protein. This will be employed to induce sensitization. Once this has been achieved, the intracellular persisting antigen will be altered by the addition of a new chemical grouping to the haptene. After this has been accomplished, the animal will again be injected with the antigen. The antibody titers will be closely followed to determine whether or not non toxic alteration of *in vivo* antigen eliminates the secondary response and eliminates sensitization of the immediate type. In like manner the response of *in vivo* antigenic alteration upon delayed sensitivity will be studied.

Major Findings:

None yet.

Significance to the Program of the Institute:

This study may lead to further knowledge of the immune process, and to a new approach to desensitization.

Proposed Course of Project:

This project will be continued on a very small scale until the better of these chemical approaches can be determined. After that, if it appears promising, it will be studied until definite positive or negative results have been obtained.

Part B included Yes [ ] No [X]

Part A

Project Title: Experimental "Allergic" Aspermatogenesis in Guinea Pigs

Principal Investigator: Dr. Joseph A. Bauer, Jr.

Other Investigators: Dr. Jules Freund

Cooperating Units: None

Man Years:

Total: 1.0
Professional: .50
Other: .50

Patient Days: None

Project Description:

Passive transfer of experimental "allergic" aspermatogenesis in inbred guinea pigs.

Objectives:

a. To study actively induced aspermatogenesis in inbred guinea pigs.

b. To explore the relationship of serum antibodies and/or cell borne immunity to the occurrence of aspermatogenesis in inbred guinea pigs.

c. To transfer aspermatogenesis passively within inbred strains of guinea pigs.

d. To study passive cutaneous anaphylaxis with immune sera to guinea pig testis preparations.

Methods Employed:

a. Aspermatogenesis is produced actively in inbred guinea pigs by immunization with various testicular preparations in combination with paraffin oil adjuvants.

b. Viable lymphoid cells and or antiserum from guinea pigs treated as above (a) are transferred to normal guinea pigs.

c. The incidence of passive aspermatogenesis in guinea pigs treated as above (b) is studied by histopathologic means.

Major Findings:

a. The passive transfer of aspermatogenesis with lymphoid cells has been successfully attained between inbred guinea pigs. Exhaustive attempts by other investigators using random bred guinea pigs failed to transfer aspermatogenesis, suggesting the advantage of tissue compatibility (lymphoid cells) as found in inbred strains for this type of transfer.

b. This successful passive transfer greatly strengthens the assumption that experimental "allergic" aspermatogenesis is of immunological nature and is a manifestation of sensitization of the delayed type.

c. Sera of animals immunized with testicular material, although incapable of producing aspermatogenesis or passive transfer, were found capable of eliciting passive cutaneous anaphylaxis.

Significance to the Program of the Institute:

Sterility in human males is often associated with azospermia or occasionally with aspermia. In the atrophic testes the histologic findings are in some respects similar to those seen in experimentally induced aspermatogenesis. Confirmation of the immune nature of aspermatogenesis by passive transfer may help to elucidate the nature of sterility in man. In addition, when compared with previous unsuccessful experiments with random bred guinea pigs, the present study emphasizes the significance of the use of inbred guinea pigs for cellular transfer experiments in immunology.

Proposed Course of Project:

Reproducibility of passive transfer will be ascertained and further studies performed.

Part B included Yes [ ] No [X]

Part A

Project Title: Basic Studies on the Chemical, Physical, and Skin Reactive Properties of Extracts of House Dust

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles is testing various house dust fractions for skin reactivity in specifically sensitive individuals.

Man Years: Patient Days: None

Total: 0.40
Professional: 0.25
Other: 0.15

Project Description:

Objectives:

1. To study the chemical and physical properties of the materials used clinically for the diagnosis and treatment of house dust allergy.

2. To try to identify the components responsible for the specific skin reactivity.

Major Findings:

1. This work is a continuation of a study of the chemistry of the skin reactive materials of aqueous house dust extracts that was initiated a number of years ago under the direction of Professor Dan H. Campbell at the California Institute of Technology. Most of the chemical work carried out since that time has involved the characterization of the polysaccharide components. A purified skin reactive polysaccharide fraction has been isolated and found to be a heterogeneous mixture of acidic polysaccharides. The chemical composition has been shown to be roughly 5% polypeptide and 95% polysaccharide, containing about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, D-mannose with lesser amounts of L-rhamnose and L-arabinose. . .

2. During the period covered by this report, a previous zone electrophoresis study of an aqueous house dust extract has been repeated, in part, and extended. The dust extract was fractionated in the starch electrophoresis experiments at a number of different pH's and the position of the color, nitrogen, total carbohydrate and uronic acid determined. One of the objectives was to try to separate the nitrogen containing components from the polysaccharides. One would predict that this would be possible if the nitrogen containing material were present as separate components in the dust extract or if they were bound to the acidic polysaccharides by salt linkages. Almost all the nitrogen has been found to remain with the carbohydrate, indicating the presence of covalent rather than ionic bonds. Some differences in the monosaccharide composition of the polysaccharides from different places on the starch block have been demonstrated.

Significance to the Program of the Institute:

1. About one third of all allergic individuals give a positive skin reaction to house dust extracts. These extracts have been used for many years in the diagnosis and treatment of house dust allergy. It is of importance to investigate the chemical nature of these materials and to find out what chemical structures are involved in the reactions.

2. A rational standardization of the potency of house dust allergen extracts might well be based on a knowledge of the chemical nature of the active materials.

Proposed Course of the Project:

1. It is proposed to complete the zone electrophoresis study of the house dust extracts at various pH's.

2. It is proposed to study the acid partial degradation products of the dust extracts by chemical and biological methods.

3. It is proposed to study the products of enzymatic degradation of the dust extracts by chemical and biological methods.

4. It is proposed to investigate the possibility of house dust allergen-antibody complex formation using sera from house dust sensitive individuals and the method of starch zone electrophoresis.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NIAID - 154
1. Laboratory of Immunology
2. Clinical Immunology
3. Pasadena, California

PART A

Project Title: Ultracentrifuge Studies of Skin Sensitizing Antibodies in Allergy

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

This work is being carried out in joint collaboration with Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles.

Man Years:

Total: 0.25
Professional: 0.25
Other: 0.0

Patient Days: None

Project Description:

Objectives:

To identify the sedimentation coefficient(s) of the antibodies of the sera of allergic individuals that are responsible for the specific skin sensitizing activity.

Methods Employed:

1. Sera from allergic individuals were fractionated by use of the preparative ultracentrifuge (Spinco Model L) and the Waugh partition cell in the analytical ultracentrifuge (Spinco Model E).

2. The fractions obtained were examined in the analytical ultracentrifuge, examined by free boundary electrophoresis and were tested for skin sensitizing activity by the Prausnitz-Kustner test.

Major Findings:

1. This work is a continuation of some experiments carried out in collaboration with Professor Dan H. Campbell at the California Institute of Technology about two years ago. At that time our data indicated that the skin sensitizing antibody was not unusually heavy; however, more recently Dr. Alec Sehon of the University of Montreal, reported experiments indicating that

the skin sensitizing antibody was heavy (S19). Because of this difference, we decided to carry out further experimental work.

2. The data obtained show that the skin sensitizing activity cannot be exclusively associated with the S19 component and that probably the S19 component is not active at all.

Significance to the Program of the Institute:

The physical properties of the skin sensitizing antibody in allergy are of fundamental importance in understanding allergic reactions. The skin sensitizing antibody is qualitatively different from ordinary precipitating antibody in that it is retained locally when injected intradermally rather than diffusing away. The process of antibody fixation to tissues is an important problem in all allergic reactions and any differences that can be demonstrated between precipitating and skin sensitizing antibody may be important in this process.

Proposed Course of Project:

The experiments underway will be completed and the results presented at the American Academy of Allergy meeting in February by Dr. Ernest Heimlich.

Part B included Yes [ ] No [X]

NIAID
Rocky Mountain
Laboratory

Estimated Obligations for FY 1959

Total:	$1,069,500
Direct:	1,069,500
Reimbursement:	0

Covering Project Reports
NIAID....170 thru 179

ROCKY MOUNTAIN LABORATORY
Hamilton, Montana

Annual Report of Program Activities----------Calendar Year 1958

NIAID-170 OFFICE OF THE DIRECTOR
Principal investigator: C. L. Larson

NIAID-171 IMMUNE PROPHYLAXIS OF MYCOBACTERIAL INFECTIONS
Principal investigator: E. Ribi
Others: C. L. Larson, T. D. Perrine

NIAID-172 IMMUNE PROPHYLAXIS OF SALMONELLA INFECTIONS
Principal investigator: E. Ribi
Others: F. G. Jarvis, C. L. Larson, K. C. Milner, T. D. Perrine

NIAID-173 STUDIES ON COLORADO TICK FEVER
Principal investigator: C. M. Eklund
Others: J. M. Brennan, W. Burgdorfer, R. K. Gerloff, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. Luoto, L. A. Thomas

NIAID-174 STUDIES ON DISEASES HAVING A RESERVOIR OF INFECTION IN THE NATURAL ENVIRONMENT
Principal investigator: J. F. Bell
Others: W. L. Jellison, D. B. Lackman, L. Luoto, M. T. McKee, C. R. Owen, H. G. Stoenner

NIAID-175 TRANSMISSION OF DISEASE AGENTS BY CERTAIN VECTORS
Principal investigator: C. B. Philip
Others: J. F. Bell, J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman

NIAID-176 ALLERGY AND IMMUNOLOGY OF FUNGAL INFECTIONS AND THE MECHANISMS OF ALLERGIC PHENOMENA
Principal investigator: S. B. Salvin
Others: J. F. Bell, R. F. Smith

NIAID-177 STUDIES ON THE ENCEPHALITIDES
Principal investigator: C. M. Eklund
Others: J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. A. Thomas

NIAID-178 INVESTIGATION OF RICKETTSIAL INFECTIONS
Principal investigator: C. B. Philip
Others: E. J. Bell, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. Luoto, R. A. Ormsbee, H. G. Stoenner

NIAID-179 VIRUSES, VIRUS COMPONENTS, AND VIRUS SURFACES
Principal investigator: B. H. Hoyer
Others: R. A. Ormsbee, D. B. Ritter

Serial No. NIAID-170
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Office of the Director

Principal Investigator: C. L. Larson

Other Investigators: None

Cooperating Units:

Man Years (calendar year 1958):
Total: 19
Professional: 1
Other: 18

Project Description:

The over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; the direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping, and maintenance activities; and the providing of scientific services as required by the research staff, including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, etc.

Part B included Yes X No

Serial No. NIAID-170

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

In press:

Lackman, D., Gerloff, R., and Philip, R.: A comparison of complement fixation and metabolism inhibition as methods of evaluating poliomyelitis antibody response. Am. J. Pub. Health.

Lackman, D., Casey, M., Philip, R., Owen, C., and Reinhard, K. R.: A comparison of influenza in the Northwest caused by A-prime and Asian strains of influenza virus. Can. J. Pub. Health.

Honors and Awards relating to this project:

Dr. C. L. Larson

Invited to serve for an additional 5-year period on the Expert Advisory Panel on Zoonoses of the World Health Organization.

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Elected to Charter Fellowship in the American Academy of Microbiology, Inc.

Serial No. NIAID-171
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Immune prophylaxis of mycobacterial infections

Principal Investigator: E. Ribi

Other Investigators: C. L. Larson and T. D. Perrine

Cooperating Units:

Man Years (calendar year 1958):
Total: 14
Professional: 2
Other: 12

Project Description:

Objectives:

Immunity in tuberculosis has not been established as a factor influencing the outcome of the disease. Debate continues regarding the merits of immunization procedures. The possibility exists that a multiplicity of antigens are produced by *Mycobacterium tuberculosis* and that many of these may produce such deleterious effects as hypersensitivity which may overshadow the beneficial effects of antigens producing resistance in the host. It is the object of this research to systematically isolate fractions of acid-fast bacilli by mild chemical and physical means, to assay them for activity in normal animals, and to test them for their ability to protect mice against tuberculosis initiated through the pulmonary tract.

Methods:

The methods employed are centered around three techniques: (1) the separation of mycobacteria primarily into cell wall and protoplasm fractions by physical means and secondarily to obtain the constituent portions of each of these fractions, (2) tests of the activity of these fractions in rabbits to determine their ability to produce dermal necrosis and to induce hypersensitivity, and (3) tests of the ability of these materials to determine if they are capable of protecting mice against infection with *M. tuberculosis* administered intranasally.

Part B included Yes X No ___

A new method has been developed for obtaining cell walls and protoplasm in quantity from acid-fast bacilli. This method employs a modified pressure cell together with cooling mechanisms devised to decrease denaturization of proteins which might occur due to heating concurrent with rupture of the organisms. With this technique it is possible to obtain as much as 60 mg of cell walls from each gram of intact cells. This yield is at least ten times that obtained with the previous procedure.

Normal rabbits given protoplasm from M. tuberculosis intradermally failed to develop primary lesions and subsequent hypersensitivity against cell walls, protoplasm, tuberculin, or P.P.D. Rabbits given cell walls, however, develop primary lesions and subsequent delayed hypersensitivity toward cell walls, protoplasm, whole cells, old tuberculin, and P.P.D.

A soluble material may be obtained from tubercle bacilli or from the spleens of animals infected with these organisms by a modification of the Ascoli procedure. This material will not immunize mice, but is specifically precipitated by serums of animals immunized with dead bacteria or their products.

Attenuated strains of M. tuberculosis which have been grown for a number of passages on liquid media will not immunize mice against infection.

The Rocky Mountain Laboratory strain of mice may be uniformly infected by intranasal instillation of dilute suspensions of the tubercle bacillus. Tubercles develop in the lungs, and the animals subsequently develop a bacteremia which can be measured by the number of organisms which can be isolated from the spleens. Animals immunized with living avirulent strains of M. tuberculosis (BCG and H37Ra) can be shown to respond to intranasal infection both by a decrease in the number of lesions which are found in the lungs and by the absence of bacteremia. More than 50% of mice given a 1:100 dilution of avirulent organisms are resistant to infection.

Significance:

The tubercle bacillus has been fractionated into its primary morphological parts and, with the apparatus recently described, sufficient material will be made available to carry out experiments with large enough doses of antigen to determine if these materials are capable of inducing immunity against pulmonary infections.

While many studies have been made on the ability of various living vaccines to prevent tuberculosis, the methods for testing immunity have not yielded results that are in all cases convincing. It would appear that the use of intranasal inoculation causes an illness in experimental animals which more nearly resembles

occurrences under natural conditions and yields results which indicate that properly prepared living vaccines will produce satisfactory immunity. These observations suggest a distinct possibility that the immunogenicity of killed organisms and fractions from these can be analyzed and that the factor responsible for inducing immunity can be identified.

Proposed course:

Attempts will be made to produce large quantities of cell walls and protoplasmic fractions from cultures of attenuated organisms grown for only one or two passages on liquid media. Mice will be immunized with large quantities of such fractions. We have previously been able to deal with doses as large as 50 µg, whereas the newer methods will allow us to give doses of about 1 mg to each animal. If immunity is produced by these fractions, attempts will be made to purify and isolate the responsible material.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ribi, E., Larson, C. L., List, R., and Wicht, W.: Immunologic significance of the cell wall of mycobacteria. Proc. Soc. Exp. Biol. & Med. 98: 263-265. June 1958.

Honors and Awards relating to this project:

Dr. E. Ribi

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Invited to participate in the Study Program in Biophysical Science, sponsored and organized by the Biophysics and Biophysical Chemistry Study Section, Boulder, Colorado.

Serial No. NIAID-172
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Immune prophylaxis of Salmonella infections

Principal Investigator: Edgar Ribi

Other Investigators: F. G. Jarvis, C. L. Larson, K. C. Milner, and T. D. Perrine

Cooperating Units:

Man Years (calendar year 1958):
Total: 10.5
Professional: 2.5
Other: 8

Project Description:

Objectives:

This program is an extension of the original investigation which resulted in the isolation of cell walls from Bacterium tularense. It has as its primary aim the characterization of biologically active fractions of Salmonella sp. according to their morphological, chemical, and biological attributes, and as its secondary aim the production of vaccines suitable for immunization of man and animals.

Methods:

The methods employed are varied, since the approach to the problem is complex. It is necessary to grow organisms on defined media in order to avoid contamination with components of the medium. Collection of cell walls and protoplasm as the initial step is controlled by continuing electron microscopic observation of the process, which involves mild chemical or physical methods. Biological examination is difficult, since the animal tests purported to be valuable in estimating immunity are not wholly acceptable. Stress is placed upon tests which will measure immunity toward infection rather than toxicity. Much of the work has been done by employing S. enteritidis as a test system, but recently S. typhosa has been studied intensely.

Part B included Yes X No

1. A test for evaluation of immunity has been developed. Experiments demonstrate that the system tests for antibacterial and not for antitoxic immunity, that immunity is specific and requires stimulation by one or more antigens specific for each organism under consideration, and that the degree of immunity is related to the quantity of antigen administered and the interval between immunization and challenge. Statistically the test has been shown to be suitable for assay of various fractions isolated from Salmonella.

2. A simple test for determining the relative potency of endotoxins derived from various Salmonella has been perfected. Identical preparations tested in mice and in the skin of normal rabbits indicate the rabbit test to be at least 100-fold more sensitive than the mouse test.

3. Lipids have been extracted from the endotoxin moiety by a variety of methods which reduce the total lipid content from 12% to 1%. The remaining portion retains its toxicity while the lipid fraction is nontoxic. The antigenicity of fractions from which lipids are removed is not decreased.

4. Further treatment of delipified materials indicates that their antigenicity and toxicity is primarily attributable to the polysaccharide portion of the endotoxin.

5. Delipified endotoxin was found to be homogeneous when studied in the optical centrifuge and to have a sedimentation constant of 18S indicating a molecular weight of about 1 million.

6. A chemically defined medium has been made for production of Vi antigen. In addition to glucose it contains certain salts, thiamine, pantothenic acid, glutamic acid, aspartic acid, and tryptophane. Yields of 2,000 to 6,000 units/ml of media can be obtained from E. coli 5396/38. Methods of harvesting and purifying Vi antigen based on extraction of organisms with ether and purification by electrophoresis have been developed. Chemical and physical studies of purified Vi antigen suggest that 2 or more chemically distinct types of Vi antigen exist and that mild hydrolysis of Vi antigen degrades the antigen with a resultant loss in certain biologic activities, including immunogenicity in mice.

Significance:

The broad implications of this program have been discussed in the previous report. The present year's activity is of importance in eliminating lipid from consideration as a necessary portion of antigens responsible for the toxic action of Salmonella and for production of antibacterial immunity. These properties appear to be related to the carbohydrate portion of a material which has a surprisingly low molecular weight (1,000,000 as compared to 8,000,000 for Shear's preparation and 20,000,000 for Westphal's endotoxin).

Development of a skin test in rabbits for assay of endotoxins decreased very considerably the amount of endotoxin required for assay purposes and consequently allows research to be performed with materials which are obtainable only in small amounts.

The assay method which has been applied to test the ability of fractions from Salmonella to produce antibacterial immunity in mice is of great value in studying immunity against these infections since it is specific, measures antibacterial rather than antitoxic immunity, and produces immune responses which are related to the dose of antigen administered to mice. These factors indicate that we are studying responses in animals which may be similar to those in man.

Vi antigen is very probably the simplest cell-wall antigen found in this group of bacteria, and it should be possible to correlate antigenicity with chemical structure by employing this material. In addition, the present results indicate that this antigen may be important in protection against typhoid fever.

Proposed course:

Much of the work done during the last year has been in the direction of determining the physical, chemical, toxic, and immunologic behavior of endotoxins. The total work indicates that the problem of immunizing man against Salmonella infections could be solved by application of the technique and methods developed during the course of the study. This direction is one which we could hardly afford to take as the skills of the group are in the laboratory sciences rather than in epidemiology and clinical medicine.

Consideration is given to the following courses which the study might take. A study of the simplest compounds required to produce immunity against infections with Gram-negative organisms could lead to the development of "synthetic antigens" which might be applied to the prevention of multiple infections. Or, the personnel, equipment, and professional attributes of the group could be applied to a study of streptococcal antigens. The great need for this type of work is attested to by the present methods which are employed for the prevention of streptococcal disease in children and Service personnel. The use of therapeutic measures rather than specific immunization to control streptococcal disease indicates that greater effort should be made to obtain materials which could be administered to selected groups.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Jellison, W. L. and Milner, K. C.: Salmonellosis (Bacillary dysentery) of fur seals. J. Wildlife Management 22: 199-200. April 1958.

In press:

Ribi, E., Milner, K. C., and Perrine, T. D.: Endotoxic and antigenic fractions from the cell wall of Salmonella enteritidis. Methods for separation and some biological activities. J. Immunol.

Serial No. NIAID-173
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Colorado tick fever

Principal Investigator: C. M. Eklund

Other Investigators: J. M. Brennan, W. Burgdorfer, R. K. Gerloff, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. Luoto, and L. A. Thomas

Cooperating Units:

Man Years (calendar year 1958):
Total: 23
Professional: 5
Other: 18

Project Description:

Objectives:

In general terms the investigations pertaining to this disease are directed toward (1) definition of the clinical syndrome occurring in man, (2) determination of the geographical limits of the disease, (3) production of a vaccine for the prevention of disease in man, and (4) examination of the ecological features of the disease in nature. This latter item includes studies of arthropods and animals which not only are hosts to the several stages of ticks but develop significant viremia when exposed to the virus by natural means.

Methods:

The techniques and methods employed are not significantly different from those employed to study other viral diseases except as they have been adapted to the needs of the particular situation.

Major findings:

1. Tissue cultures are as efficient as suckling mice for the isolation and identification of Colorado tick fever virus from man and other sources. In 196 attempts to isolate virus

Part B included Yes X No

from blood clots of human beings suspected of having CTF, 62 isolations were made in mice and 68 in tissue cultures.

2. A tissue-culture neutralization test for CTF antibodies was developed employing KB cells in the system. The tissue-culture system was as sensitive and specific for the detection of antibodies as the test in suckling mice. An example of its use are the findings obtained from examination of serums from certain selected groups: 32% of sheepshearers, 6% of mammalogists, and 5% of persons in Idaho had antibodies, while no persons in Turkey or Alaska had antibodies.

3. A complement-fixation test for the diagnosis of CTF was devised. It is sufficiently sensitive to be employed as a routine method of diagnosis. Antibodies reacting in complement-fixation tests are slow in developing and are not detectable until about a week after neutralizing antibodies may be noted.

4. Porcupines (Erethizon dorsatum) develop viremia following administration of virus either by intraperitoneal inoculation of virus or by the bites of infected ticks. Viremia was as high as 10^5 mouse LD_{50} of whole blood.

5. Ecology of CTF. A 5-year study of ticks in Mill Creek Canyon shows that the incidence of infected ticks present in limited biotic areas may vary from 0.3% to 11.3% in any one year and the incidence in a single area may vary within these limits from year to year. In Lost Horse Canyon the incidence of infection among ticks taken in 5 different areas varied from 0% to 16.1%. Golden-mantled ground squirrels appear to be the preferred host for immature ticks and they are likewise found to be more frequently infected than Columbian ground squirrels. Among the former species, the incidence of viremia was 45.9% and among the latter it was 12.9%. Viremia was also detected in chipmunks, pine squirrels, and deer mice. Virus titers of $10^{3.3}$ mouse MLD_{50}/ml blood were noted, and viremia was demonstrated to persist for 20 days in a deer mouse observed in the field.

6. Clinical aspects: Serious illness has been recorded in 8 patients. These include 5 with encephalitis, 2 with severe bleeding, and 1 with both severe bleeding and central nervous system symptoms. Serological surveys on serums collected in Colorado, Wyoming, and Idaho indicate 14.9%, 12.8%, and 7.0% of individuals have antibodies against this virus.

7. Development of vaccine. Vaccine was prepared from the brains of suckling mice infected with CTF virus by inactivation with formaldehyde. While the vaccine was capable of preventing viremia in adult mice, it has produced no antibodies in a group of human volunteers who received 2 doses of vaccine at weekly intervals. No untoward reactions were noted in these individuals.

Development of a complement-fixation test for the diagnosis of CTF makes available a tool which can be employed in State and other local health laboratories on a routine basis. Tissue-culture techniques for isolation and identification of the virus have likewise been placed in the hands of diagnostic laboratories. These methods should greatly increase the efficiency of local units to recognize the presence of the disease in this area.

Rapid strides were made in understanding the ecology of this disease. Recognition of the fact that ticks do not transmit infection to succeeding generations aided in directing studies of animal reservoirs. It has been established that many rodents are capable of becoming infected and of maintaining a high level of viremia for considerable periods of time. These observations suggest that rodent control is of primary importance in attempts to control human infection.

The large numbers of cases of CTF occurring annually and the severity of illness noted in many individuals emphasize the need for a vaccine. The success obtained in experimental animals suggests that a vaccine can be developed.

Proposed course:

The profitable studies relating to ecology will be continued. There is evidence that limited biotic areas have vast differences in the incidence of infection in rodents and arthropods, and studies to determine the factors responsible for these differences will be continued.

There are many factors which must be considered in preparing a vaccine from CTF virus. The agent is extremely sensitive to manipulation, and the methods which are employed to destroy viable virus may be so vigorous as to destroy antigenicity. Studies of methods of producing large quantities of virus and of producing nonviable but antigenic vaccine will be intensified.

Serial No. NIAID-173

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Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eklund, C. M., Kohls, G. M., and Jellison, W. L.: Isolation of Colorado tick fever virus from rodents in Colorado. Science 128: 413. Aug. 1958.

Pickens, E. G. and Luoto, L.: Tissue culture studies with Colorado tick fever virus. I. Isolation and propagation of virus in KB cultures. J. Inf. Dis. 103: 102-107. July-Aug. 1958.

In press:

Burgdorfer, W.: Colorado tick fever. The behavior of CTF virus in the porcupine. J. Inf. Dis.

Gerloff, R. K. and Eklund, C. M.: A tissue culture neutralization test for Colorado tick fever antibody and use of the test for serologic surveys. J. Inf. Dis.

Rozeboom, L. E. and Burgdorfer, W.: Development of Colorado tick fever virus in the Rocky Mountain wood tick, Dermacentor andersoni. Am. J. Hyg.

Honors and Awards relating to this project:

Dr. L. Luoto

Appointed Chairman, Career Public Health Veterinarians and Practioners. First Institute of Veterinary Public Health Practice, School of Public Health, Univ. of Mich., Ann Arbor, Oct. 6-9, 1958.

Serial No. NIAID-174
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on diseases having a reservoir of infection in the natural environment

Principal Investigator: J. F. Bell

Other Investigators: W. L. Jellison, D. B. Lackman, L. Luoto, M. T. McKee, C. R. Owen, and H. G. Stoenner

Cooperating Units:

Man Years (calendar year 1958):
Total: 13
Professional: 4
Other: 9

Project Description:

Objectives:

Work done under this category is directed toward the ecological aspects of diseases which are transmissible from animals to man and toward laboratory studies of the organisms and animals involved.

Methods:

The methods employed in the past year are not distinctly different from those ordinarily used in this type of investigation.

Major findings:

1. Rabies virus was isolated from a single bat which had attacked a hunter. In this instance virus was isolated from mice bitten by the bat. As far as we are aware this is the first time virus has been transmitted to experimental animals by the bite of bats.

2. Throughout the West there was a tremendous increase in the population of Microtus sp. during 1957 and 1958. Studies were initiated near Klamath Falls, Oregon, and contiguous areas in California. During the course of these studies, effort was

Part B included Yes X No

expended primarily to determine what factors might serve to limit the population of Microtus. It was apparent that circumscribed outbreaks of tularemia serve to eliminate large numbers of animals. Transmission appeared to be on the basis of cannibalism. A total of 157 cultures of Bacterium tularense was isolated from mice and 21 from natural waters. These organisms were demonstrated to be only moderately virulent.

3. Since the volume of work involved in determining the cause of death of mice was tremendous, the Ascoli test was employed and demonstrated to be a highly satisfactory tool for field studies of tularemia.

4. In addition to these findings, an interesting disease, known locally as "big foot," was studied. This was found to be caused by a Group A streptococcus. A similar syndrome has been described as being caused by either a virus or a mycobacterium, but this disease was differentiated from the previously described ones.

5. Cell walls and protoplasmic fractions of Pasteurella pestis have been obtained. The protoplasmic material failed to produce lesions in the skin of normal rabbits but was toxic for mice, whereas the cell wall fraction was capable of producing skin lesions in rabbits but was not toxic for mice.

6. Brucella neotomae and Br. suis have a close immunological relationship, and cross protection experiments show that protection is partially dependent upon the relative susceptibility of the host to the two organisms.

Significance:

It is particularly fortunate that the strains of tularemia organisms which have been isolated recently from animal hosts and natural waters in California and Oregon are of only moderate virulence. If highly virulent organisms had been present in these areas, a considerable number of human cases of tularemia would have been encountered. The knowledge that we were dealing with the former type of pathogen led us to refrain from use of vaccines for the prevention of tularemia in man.

The demonstration that naturally infected bats may transmit rabies to experimental animals by bite suggests the mechanism whereby rabies is maintained among bats in nature and the mechanism whereby transmission to man and other animals may occur.

Proposed course:

It is proposed to continue and expand banding and trapping activities which only this year were initiated with the bat population

in Montana. Studies will be made of the incidence of infection in these animals and on the mode of transmission of the infection. Attempts are being made to establish a colony of bats for studies of pathogens.

Studies will be continued on immunization against rabies by employing methods to increase the amount of virus available as an antigen and by purifying preparations to be used for immunization.

Studies of vaccines prepared from P. tularensis and P. pestis will be continued.

A study of the susceptibility of swine to infection with Br. neotomae is planned with a view toward use of this organism to prevent Br. suis infections in swine.

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Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Calero M., C., Reinhard, K. R., and Owen, C. R.: Leptospirosis of man in the Isthmus of Panama. Am. J. Trop. Med. & Hyg. 6: 1054-1060. Nov. 1957.

Jellison, W. L. and Hadlow, W. J.: Parasitic pulmonary granuloma in the Townsend mole. Proc. Helminthol. Soc. Wash. 25: 67-70. Jan. 1958.

Jellison, W. L. and Parker, H.: Survival of the pathogenic fungus, Coccidioides immitis, under CO_2 refrigeration. Proc. Symposium on Coccidioidomycosis, Phoenix, Ariz., P.H.S. Publ. No. 575, pp. 30-31. Dec. 1957.

McKee, M. T., Bell, J. F., and Hoyer, B. H.: The culture of Clostridium botulinum type C with controlled pH. J. Bact. 75: 135-142. Feb. 1958.

Stoenner, H. G. and Maclean, D.: Leptospirosis (ballum) contracted from Swiss albino mice. AMA Arch. Inter. Med. 101: 606-610. Mar. 1958.

Stoenner, H. G., Grimes, E. F., Thrailkill, F. B., and Davis, E. J.: Elimination of Leptospira ballum from a colony of Swiss albino mice by use of chlortetracycline hydrochloride. Am. J. Trop. Med. & Hyg. 7: 423-426. July 1958.

Jellison, W. L.: Haplomycosis in Japan and Africa. Mycologia 50: 580-583. July-Aug. 1958.

Bell, J. F., Owen, C. R., and Jellison, W. L.: Group A streptococcus infections in wild rodents. J. Inf. Dis. 103: 196-203. Sept.-Oct. 1958.

In press:

Jellison, W. L., Bell, J. F., Vertrees, J. D., Holmes, M. A., Larson, C. L., and Owen, C. R.: Preliminary observations on diseases in the 1957-58 outbreak of Microtus in western United States. Proc. 23d N. A. Wildlife Conference.

Bell, J. F., Jellison, W. L., Owen, C. R., and Larson, C. L.: Applicability of the Ascoli test to epizootic tularemia in wild rodents. J. Wildlife Management.

Jellison, W. L., Stoenner, H. G., and Berg, G. M.: Water-borne leptospirosis among Indians on the Standing Rock Indian Reservation, North Dakota and South Dakota, during the summer of 1956. Rocky Mtn. Med. Jour.

Honors and Awards relating to this project:

Dr. J. F. Bell

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Dr. W. L. Jellison

Secretary of the International Northwest Conference on Diseases in Nature Communicable to Man.

Dr. H. G. Stoenner

Appointed to the Committee on Leptospirosis of the U. S. Livestock Sanitary Association.

Serial No. NIAID-175
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Transmission of disease agents by certain vectors

Principal Investigator: C. B. Philip

Other Investigators: J. F. Bell, J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, and D. B. Lackman

Cooperating Units:

Man Years (calendar year 1958):
Total: 11.5
Professional: 2.5
Other: 9

Project Description:

Objectives:

The objective of this program is to study the ecology of diseases transmissible to man by insect vectors and to obtain information on the mechanisms whereby these insects are able to serve efficiently as vectors. The ecological approach to insect-borne diseases, coupled with experimental observations directed toward solution of problems arising from field studies, produces results which may be applied to control of these diseases.

Methods:

In general, the methods previously employed or developed in this laboratory have been applied. Fluorescent microscopy has been used to determine whether or not Colorado tick fever virus may be transmitted transovarially by the tick (Dermacentor andersoni).

Major findings:

1. Tick paralysis. Hamsters (Cricetus auratus) have been found to be sensitive to the agent causing paralysis, and a definite syndrome develops following feeding by affected ticks. Only adult ticks are capable of inducing illness. It has been

Part B included Yes X No

possible to cause illness in hamsters by the bite of adult ticks (F_1 generation) reared from a tick responsible for disease in man. The toxic material appears to affect the central nervous system, although there is some involvement of the peripheral nerves.

2. Colorado tick fever. It has been shown that this virus does not have a developmental cycle in ticks comparable to that of yellow fever virus in mosquitoes. The quantity of virus ingested by larvae or nymphs remains relatively constant during the quiescent stage but increases significantly following molting to the nymphal or adult stage. Further studies on the transovarial transmission of virus by employing individual infected ticks revealed that in no instance was virus present in eggs, larvae, or adults descended from over 40 females known to be infected at the time when oviposition was completed. D. occidentalis has been shown to be infected in nature, while Ixodes pacificus appears to be free of infection with this virus.

3. Trombiculids: Four new genera of chiggers have been described. Information on the host relationships, distribution, speciation, and general ecology of chiggers has been greatly expanded.

4. Ixodidae: A new species, Amblyomma imitator, has been described and distinguished from A. cajennense. The confused findings relative to the transmission of spotted fever by ticks of this genus is thus clarified. The tick vector responsible for transmission of Kyasanur Forest Disease was identified from collections studied for the Rockefeller Virus Laboratory, Poona, India.

5. Mosquitoes: Cubicles for rearing colonies of mosquitoes have been designed, constructed, and placed in operation.

Significance:

The demonstration that hamsters are very sensitive to the agent responsible for causing tick paralysis makes available an experimental animal useful for studying the etiology and pathogenesis of this most interesting disease and also for determining the magnitude of the reservoir which exists in ticks in nature. Research has been hampered not only by previous lack of a suitable experimental host but by the lack of a colony of arthropods with a known incidence of disease. Present studies indicate that a colony known to be affected may be developed by rearing progeny from a single tick taken from a host suffering from paralysis. Rapid strides toward an understanding of the pathogenesis of this illness may be expected now that an experimental animal and a colony of known capabilities have been provided.

An exact knowledge of the behavior of Colorado tick fever virus in its insect host serves to describe the nature and extent of the potential reservoir of infection and to suggest methods of control of the infection. Definite evidence for the absence of hereditary transmission of the virus in D. andersoni establishes that animal hosts are primarily responsible for maintenance of the disease in nature. Further mention of the ecology of Colorado tick fever has been made in a previous section of the report.

An example of the extreme value to medical science of facilities and personnel skilled in taxonomy is afforded by our identification of the insect vector of Kyasanur Forest Disease which aided in establishing the ecology of the disease and in suggesting methods for the prevention of disease in man.

Proposed course:

Increased effort will be made to establish a colony of ticks which will, with reasonable certainty, be capable of producing tick paralysis in hamsters. The symptoms and pathology of the disease in hamsters will be established and, in conjunction with pharmacologists at the University of Utah, the pharmacology and physiology of the disease will be investigated. The production of the toxic agent in feeding adult female ticks will be studied in order to determine the factors governing the presence and the increase in quantity of the material.

Further studies of Colorado tick fever virus in ticks will be made especially in order to determine the mutual influence of this virus and spotted fever rickettsiae upon each other when both organisms are present in a tick.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Brennan, J. M.: Revival of Crotiscus Ewing, 1944, and descriptions of four new species of the genus from Peruvian rodents (Acarina: Trombiculidae). J. Parasitol. 43: 673-680. Dec. 1957.

Kohls, G. M.: Ixodes downsi, a new species of tick from a cave in Trinidad, British West Indies (Acarina-Ixodidae). Proc. Ent. Soc. Wash. 59: 257-264. Dec. 1957.

Kohls, G. M.: Ticks (Ixodoidea) of Borneo and Malaya. In Malaysian Parasites XVI-XXXIV. Studies from the Inst. for Med. Res., Federation of Malaya, No. 28. Edited by J. R. Audy. XVIII, pp. 65-94. The Caxton Press Ltd., Kuala Lumpur, Malaya. 1957.

Kohls, G. M.: Insects of Micronesia. Acarina: Ixodoidea. INSECTS OF MICRONESIA published by Bernice P. Bishop Museum, Honolulu, Hawaii. 3: 85-104. 1957.

Sargent, H. G.: A technique for killing and preserving ticks for study. Ward's Nat. Sci. Bull. 31: 47. 1958.

Brennan, J. M.: Beamerella and Kaaia, two new genera of chiggers from South America (Acarina, Trombiculidae). J. Kan. Ent. Soc. 31: 71-75. April 1958.

Philip, C. B.: Five new species of Tabanidae (Diptera) from Mexico and Brazil. J. Kan. Ent. Soc. 31: 177-184. April 1958.

Philip, C. B.: Descriptions of new Neotropical Tabanidae mostly in the California Academy of Sciences (Diptera). Pan-Pac. Ent. 34: 63-76. April 1958.

Kohls, G. M.: Amblyomma imitator, a new species of tick from Texas and Mexico, and remarks on the synonymy of A. cajennense (Fabricius) (Acarina--Ixodidae). J. Parasitol. 44: 430-433. Aug. 1958.

Philip, C. B., Hughes, L. E., and Darrow, D. I.: Experimental transmission of yellow fever virus by oriental mosquitoes. Proc. 10th Intern. Cong. Ent. Montreal, Aug. 17-25, 1956. 3: 587-592. Dec. 1958.

Philip, C. B.: A helminth replaces the usual arthropod as vector of a rickettsialike disease. Proc. 10th Intern. Cong. Ent. Montreal, Aug. 17-25, 1956. 3: 651-653. Dec. 1958.

In press:

Brennan, J. M. and Jones, E. K.: Keys to the chiggers of North America with synonymic notes and descriptions of two new genera (Acarina: Trombiculidae). Ann. Ent. Soc. Amer.

Brennan, J. M. and Loomis, R. B.: A review of the reptile chiggers, genus Fonsecia (Acarina, Trombiculidae), with descriptions of two new American species. J. Parasitol.

Brennan, J. M.: Synonymy of Odontacarus Ewing, 1929, and Acomatacarus Ewing, 1942, with redescriptions of O. dentatus (Ewing) and O. australis (Ewing), also descriptions of three new species from southern United States (Acarina: Trombiculidae). Ann. Ent. Soc. Amer.

Hughes, L. E. and Philip, C. B.: Experimental tick paralysis in laboratory animals and native Montana rodents. Proc. Soc. Exp. Biol. & Med.

Jellison, W.: Fleas and disease. Ann. Rev. Ent.

Philip, C. B.: New records of Tabanidae in the Antilles. Supplemental report. Am. Mus. Novitates.

Rush, W. A., Brennan, J. M., and Eklund, C. M.: A natural hibernation site of the mosquito, Culex tarsalis Coquillett, in the Columbia River Basin, Washington. Mosq. News.

Brennan, J. M.: A Croesus among insects. Nat. History.

Kohls, G. M.: Ixodides. Encyclopedia of Science and Technology. McGraw-Hill Book Co.

Philip, C. B.: Some Tabanidae (Diptera) from the Philippines with records of other species in the Far East. Fieldiana: Zoology.

Philip, C. B. and Mackerras, I. M.: On some Eastern Asiatic Chrysopinae (Diptera: Tabanidae). Philippine J. Sci.

Philip, C. B. and Aitken, T. H. G.: Records of Tabanidae from Sardinia and Corsica. Memorie della Societa Entomologica Italiana and Centro di studio di Entomologia alpina e forestale del Consiglio Nazionale delle Ricerche.

Philip, C. B.: Further records of Neotropical Tabanidae (Diptera) mostly from Peru. Proc. Calif. Acad. Sci.

Honors and Awards relating to this project:

Dr. C. B. Philip

Appointed as one of 5 official U. S. delegates to the International Commission on Zoological Nomenclature by the American Entomological Society and as a representative of this Society and the American Society of Parasitologists to the XVth International Congress of Zoology held in London, England.

Appointed as one of 5 official U. S. delegates to the International Commission on Bacteriological Nomenclature by the Society of American Bacteriologists.

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Serial No. NIAID-176
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Allergy and immunology of fungal infections and the mechanisms of allergic phenomena

Principal Investigator: S. B. Salvin

Other Investigators: J. F. Bell and R. F. Smith

Cooperating Units:

Man Years (calendar year 1958):
Total: 11
Professional: 3
Other: 8

Project Description:

Objectives:

(1) To study the basic antigen-antibody mechanism responsible for delayed hypersensitivity, (2) to study the relation between delayed hypersensitivity and the formation of circulating antibody, and (3) to determine the effect of delayed hypersensitivity on susceptibility to chronic infectious disease.

Methods:

The methods employed are the classical immunological procedures, both biological and chemical in nature. In addition to the use of such purified materials as egg albumin and diphtheria toxoid, studies are being pursued employing heterologous tissues. The chronic infectious processes under consideration include histoplasmosis and infections due to Candida albicans.

Major findings:

1. It has been shown that guinea pigs develop delayed hypersensitivity prior to the appearance of circulating antibody. Hypersensitivity to crystalline diphtheria toxoid is manifested about 5 days after sensitization and continues until about the 11th day. Following this, circulating antibody and immediate hypersensitivity reactions prevail.

Part B included Yes X No

2. Irradiation of guinea pigs with 200 to 300r of X-irradiation 18 to 24 hours prior to sensitization causes a striking delay in the appearance of antibodies. Antibodies are not detected until about the 21st day. There is, however, no change in the manifestations of delayed hypersensitivity. If irradiation was administered 12 to 24 hours after administration of toxoid, circulating antibodies develop at the normal time. Thus, the mechanisms responsible for the induction of delayed and immediate hypersensitivity may be differentiated on the basis of their reaction to the administration of radiation.

3. A highly purified complex has been obtained from the yeast phase of Histoplasma capsulatum. This material may be used as a skin test antigen. It is also capable of increasing the resistance of mice to lethal challenge. It does not, however, fix complement in the presence of antibody from infected animals or man. This material has been shown to be a protein-carbohydrate complex, and the antigenic behavior associated with hypersensitivity seems to be related primarily to the protein portion of the molecule.

4. Crude antigens obtained from C. albicans render mice more susceptible to challenge with the specific organism than are normal animals. This phenomenon is strikingly similar to the "enhancement process" which has been described with regard to tissue transplants.

5. Purified antigens have been obtained from Trichophyton rubrum and are being employed in human volunteers to determine whether increased or decreased hypersensitivity would correspondingly increase or decrease the invasiveness of the pathogen.

6. The factor has been obtained from guinea pig leucocytes which is lethal to normal animals. Animals develop nystagmus, paralysis, and ultimately die. After injection of disrupted cells, the factor appears to be in the sedimentable portions of the disrupted leucocytes.

Significance:

The role of hypersensitivity in certain infectious diseases and the allergic diseases of man has not been fully elucidated. An understanding of the mechanisms responsible for delayed and immediate hypersensitivity and the relation of these mechanisms to each other would make it possible to more intelligently diagnose and treat those conditions in which there is an underlying allergic disturbance. The discovery that a purified antigen is capable of producing not only delayed but immediate hypersensitivity and the fact that delayed hypersensitivity precedes immediate sensitivity suggest that the host itself responds to the antigen in different ways. This is further substantiated by the fact that treatment with X-rays delays the occurrence of immediate hypersensitivity but does not in any way change the course of delayed hypersensitivity.

Discovery of a purified antigen from H. capsulatum makes it possible to perform surveys in humans with an antigen which is specific and which does not show cross reactions with other organisms such as Blastomyces dermatitidis. It is also possible that with selected groups this material might be employed to produce immunity against the disease.

The interesting finding of an "enhancement factor" in C. albicans may explain failures to protect animals and man against disease and may also explain why certain diseases become chronic.

Proposed course:

Studies will be continued on the basic mechanisms of hypersensitivity. Experiments are planned to determine the initial effect of antigen on the immune process and the type of antibody which may be concerned in delayed hypersensitivity. Attempts will also be made to determine what fractions of the globulin content of serum are concerned in the immune reactions following administration of antigens.

Animals immunized with various antigenic fractions of H. capsulatum will be infected with spores via the pulmonary route in order to obtain a better understanding of the pathogenesis of chronic histoplasmosis. Further studies on the relation of hypersensitivity to infections caused by the dermatophytic fungi will also be continued and expanded. The general concept underlying the studies of histoplasmosis and infections due to C. albicans is the belief that hypersensitivity and resistance can offset one another during infection, depending upon which antigens are most active in the host, and thus influence the outcome of the disease.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salvin, S. B.: Occurrence of delayed hypersensitivity during the development of Arthus-type hypersensitivity. J. Exp. Med. 107: 109-124. Jan. 1958.

Salvin, S. B.: Serology and acquired resistance. Proc. Histoplasmosis Seminar, pp. 85-89. Feb. 11, 1958.

In press:

Salvin, S. B.: The influence of leucocytes from sensitized mice on resistance to Histoplasma capsulatum. Am. J. Hyg.

Salvin, S. B.: Delayed-type hypersensitivity and antibody formation--a discussion. In Mechanisms of Hypersensitivity, Little, Brown & Co., Boston, Mass.

Salvin, S. B.: Resistance of animals and man to histoplasmosis. In Histoplasmosis edited by Dr. Henry C. Sweany.

Salvin, S. B. and Smith R. F.: Delayed hypersensitivity in the development of circulating antibody. II. The effect of X-irradiation. J. Exp. Med.

Honors and Awards relating to this project:

Dr. S. B. Salvin

Courtesy appointment as lecturer at Montana State University, Missoula, Montana

Serial No. NIAID-177
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on the encephalitides

Principal Investigator: C. M. Eklund

Other Investigators: J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, and L. A. Thomas

Cooperating Units:

Man Years (calendar year 1958):
Total: 17
Professional: 3
Other: 14

Project Description:

Objectives:

The objectives of this program are to study the natural history of the arthropod-borne encephalitides and the behavior of the virus, hosts, and vectors under experimental conditions.

Methods:

Methods employed are those commonly used for investigations of these agents, except for those modifications adapted to our particular needs.

Major findings:

Isolations of both WEE and St. Louis viruses were made from mosquitoes in Council, Idaho, but only WEE virus from mosquitoes in North Dakota. Antibody surveys in Idaho show that many persons have been exposed to both agents, while in North Dakota persons are exposed only to WEE virus.

Culex tarsalis mosquitoes may, after ingestion of a blood meal, seek a carbohydrate meal, develop fat bodies, and hibernate.

Garter snakes (Thamnophis spp.) are readily infected with WEE virus by the bite of mosquitoes and continue to display viremia for a long period of time.

Part B included Yes X No

Snakes given EEE virus continue to circulate virus for at least 21 days with $10^{-5.5}$ to $10^{-6.3}$ M.L.D. of virus per ml of blood on the 7th day after inoculation.

St. Louis and Japanese B viruses do not appear to be as infectious for snakes as those previously mentioned, but virus was detected in a few animals on the 10th and 11th days after infection.

Significance:

The results of much work by many investigators still do not enable us to determine the factors responsible for the maintenance of these viruses in nature. While it is evident that C. tarsalis is the vector, there is little knowledge of the mechanisms of over-wintering and of the factors responsible for the spread of the virus. The discovery that mosquitoes which have had a blood meal can subsequently ingest a carbohydrate meal and go into hibernation still leaves open the question of the role of the mosquito as the over-wintering agent.

It is also possible that a cold-blooded animal such as a snake may be an efficient host of the virus. Garter snakes develop persistent and high viremia when infected with WEE and St. Louis virus which warrants their consideration as significant factors in the maintenance of infection in the field.

Proposed course:

Field studies will be continued to observe the incidence of virus in arthropods, animal hosts, sentinel animals, and man. Emphasis will be placed upon the factors responsible for maintenance of virus during various seasons and upon those responsible for fluctuation in the presence of virus.

Studies of snakes will be continued with special emphasis on the influence of hibernation upon viremia, the relations existing between infected snakes and infected mosquitoes during the over-wintering period and at the time these reptiles and insects emerge from hibernation.

By employing complement-fixation reactions, the taxonomic position of trivitattus and inornata viruses will be established, and serological studies will be made to determine if antibodies are present among residents of areas from which these viruses were isolated.

Attempts are being made to develop antigens which will serve as efficient vaccines against WEE.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Luoto, L. and Pickens, E. G.: Tissue cultures of KB epithelial cells for poliomyelitis virus tests. Pub. Health Rep. 73: 541-544. June 1958.

Eklund, C. M., Bell, E. J., and Gerloff, R. K.: Poliomyelitis in Idaho after use of live virus vaccine. Pub. Health Rep. 73: 637-647. July 1958.

Sunada, K., Gerloff, R. K., Brock, D., Hopke, I., and Klotz, A. W.: Observations of the potency and safety of poliomyelitis vaccine (Salk type) currently used. AMA J. of Diseases of Children 96: 125-130. Aug. 1958.

Gerloff, R. K. and Larson, C. L.: Serologic responses of Montana children to commercial poliomyelitis vaccine. Am. J. Hyg. 68: 74-80. July 1958.

In press:

Eklund, C. M., Hadlow, W. J., Pickens, E., and Gerloff, R. K.: Technics of value for detecting poliovirus in vaccine lots used in Idaho during 1955. Pub. Health Rep.

Gerloff, R. K., Larson, C. L., and Glesne, L. R. B.: Naturally occurring poliomyelitis antibody in Hamilton, Montana. Pub. Health Rep.

Honors and Awards relating to this project:

Dr. D. B. Lackman

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Elected to Charter Fellowship in the American Academy of Microbiology, Inc.

Dr. C. M. Eklund

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Serial No. NIAID-178
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigation of rickettsial infections

Principal Investigator: C. B. Philip

Other Investigators: E. J. Bell, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. Luoto, R. A. Ormsbee, and H. G. Stoenner

Cooperating Units:

Man Years (calendar year 1958):
Total: 11.5
Professional: 3.5
Other: 8

Project Description:

Objectives:

The objectives of this program are to study rickettsiae and the diseases caused by them. Rickettsiae are studied as organisms; their metabolism, their relations to one another, and their ability to produce disease are included in these investigations. In addition, the epidemiology of such rickettsial diseases as Q fever is under consideration.

Q fever is a problem of considerable magnitude to veterinary and human public health and its widespread occurrence throughout the United States, both in animal hosts and human beings, is again becoming recognized.

Methods:

The methods which are being employed are primarily those of epidemiologic or biologic character. Application of column chromatography has enabled us to purify rickettsial preparations.

Major findings:

1. It has been observed that Siberian tick typhus organisms belong to the spotted fever group of rickettsiae and are closely

Part B included Yes X No

related to Rickettsia rickettsii. This is the first time that R. rickettsii has been isolated from the Eastern Hemisphere.

2. The rickettsial suspensions, partially purified by modification of the method of Bovarnick and Snyder, may be purified by subjecting suspensions to chromatography on ECTEOLA columns. Suspensions of R. prowazekii, R. rickettsii, and Coxiella burnetii may be absorbed to and eluted from these columns without a decrease in the number of viable organisms. Soluble antigens from R. prowazekii and R. rickettsii may also be obtained in a high degree of purity without loss in antigenicity.

3. The epidemiology of Q fever in Idaho was studied. There have been 131 cases since 1951. Eighty-seven of these have occurred during the present year. Most of the cases occur among individuals 20 to 69 years of age, 90% occurred among males, and about 70% had onset during March, April, and May.

In 1951, only 1% of dairy herds in southcentral Idaho were found to be infected with C. burnetii, but in 1958 at least 15% of the herds showed evidence of infection. This represents a potential source of human infection and may account for the greatly increased number of cases in Idaho during the present year.

4. Cooperative studies with various State health departments, medical schools, and veterinary schools indicate that Q fever infections in man and animals are occurring throughout the country. To date, 10 new States from various parts of the country have reported infections. Many States have reported between 10% to 20% of herds to be infected.

5. C. burnetii was isolated from milk samples obtained from New Jersey and Ohio, as well as from Montana. There appear to be considerable differences between strains of Q fever isolated in various parts of the country. The virulence of these strains may contribute to the epidemiology of the disease as presently observed.

Significance:

For a number of years studies on the metabolism of rickettsiae have been performed by numerous investigators who assumed they were dealing with pure preparations of rickettsiae. Much of the work on the enzyme systems of the rickettsiae may be open to question, since it has been demonstrated by electron microscopy that the methods employed in purification do not free the rickettsia of host materials. By employing chromatography it has been possible to obtain suspensions of organisms showing no contamination with host material and, in consequence, the problem of rickettsial metabolism should be reinvestigated. The use of this simple procedure will facilitate work on rickettsial metabolism.

The widespread occurrence of Q fever in the United States and in southern Canada shows this infection to be present in animals throughout the country, constituting a potent source of infection in humans. The presence of Q fever in cattle makes it imperative that laboratory and field studies be continued in order to determine the best methods for control of this disease in both human and animal populations.

Proposed course:

During the forthcoming year, it is planned to continue epidemiological investigations of Q fever throughout the United States and to intensively study the disease in Idaho. It is not clear what animal constitutes the source of infection to humans in Idaho, even though there is a great increase in the number of infected dairy herds. Epidemiological evidence does not incriminate cattle as the source of human infection.

Another problem to be studied is the ability of Q fever organisms to develop resistance to antibiotics by residence in a host which is being fed supplements containing antibiotics. Such studies are suggested by the failure of many patients in Idaho to respond to antibiotic therapy.

Studies will be continued with chromatographic purification of rickettsiae and, following harvesting of purified suspensions, metabolic studies will be carried out to determine whether all the enzyme systems previously thought to be associated with rickettsiae are indeed associated with rickettsiae and not with contaminating material from host tissues. On the basis of results obtained from enzyme studies attempts will be made to constitute a cell-free medium for the growth of rickettsiae.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Philip, C. B.: Evidence of masking and latency in the fields of insect and helminth microbiology. Proc. Symposium on Latency and Masking in Viral and Rickettsial Infections, Univ. of Wisconsin, Sept. 4-6, 1957. Burgess Pub. Co., Minneapolis, Minn. pp. 88-106. 1958.

Luoto, L.: Rickettsial diseases of animals transmissible to children. Pediatrics 22: 384-387. Aug. 1958.

Weller, T. H., Witton, H. M., and Bell, E. J.: The etiologic agents of Varicella and Herpes zoster: Isolation, propagation, and cultural characteristics in vitro. J. Exp. Med. 108: 843-868. Dec. 1958.

In press:

Luoto, L.: The epidemiology of Q fever in the United States. Am. J. Pub. Health.

Honors and Awards relating to this project:

Dr. R. A. Ormsbee

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Dr. H. G. Stoenner

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

Serial No. NIAID-179
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Viruses, virus components, and virus surfaces

Principal Investigator: B. H. Hoyer

Other Investigators: R. A. Ormsbee and D. B. Ritter

Cooperating Units:

Man Years (calendar year 1958):
Total: 9.5
Professional: 2.5
Other: 7

Project Description:

Objectives:

The primary purpose of this program is to study the life cycle of mammalian viruses and to examine their components and surface characteristics at various stages of reproduction. Ancillary to the main thesis is the proposition that viral proteins, being noninfectious prior to association with nucleic acid to form a nucleo-protein complex, can be harvested in satisfactory yields as nonviable, antigenic material suitable for immunization purposes. Studies are made with polioviruses because they are easily adaptable to laboratory procedures.

Methods:

The methods used are of interest primarily because poliovirus is labeled with P^{32} to identify the virus and the virus nucleic acid and because column chromatography is employed to separate the virus and its component fractions. Otherwise, the methods are those ordinarily employed in virological and biochemical research.

Major findings:

1. Poliovirus has been labeled with P^{32} so that the nucleic acid portion of the virus particle and, concomitantly, the virus itself may be identified.

Part B included Yes X No

2. Purified poliovirus has been obtained by the following method: DEAE eluate ⟶ centrifuge 100,000G 90' ⟶ resuspend pellet in distilled water ⟶ centrifuge 10,000G 20' ⟶ supernatant centrifuged 100,000G 90' ⟶ pellet contains extremely pure poliovirus with tendency to form crystalline aggregates. Preparations obtained by this method have been subjected to electron microscopy, and it is apparent that this material is pure enough to be crystallizable.

3. Poliovirus prepared by this method agrees in all respects in degrees of purity with that obtained by Schwerdt and Schaffer in their studies on crystallized type 2 poliovirus.

4. Phosphorus-containing compounds and proteins from normal cells are not identical with phosphorus compounds and proteins obtained from infected cells. These normal cell constituents can be separated from the viral materials by centrifugation. By employing TEAE-cellulose columns rather than DEAE columns it is possible to separate normal cell constituents from viral proteins.

5. Virus has been treated with 5.2M urea and dissociated into a protein component and a nucleic acid component. Complete dissociation of the virus is obtained with 8M urea. This treatment, however, so adversely affects the poliovirus protein that it is no longer capable of functioning as an antigen. Material obtained from virus treated with 5.2M urea appears to be antigenic.

6. Dissociated protein is eluted from columns in the same way as whole virus, and it is therefore postulated that the column behavior of poliovirus is determined by its protein coat.

7. When poliovirus is treated with formaldehyde and subjected to chromatography, types 1 and 3 poliovirus do not change their column characteristics, while type 2 virus changes its elution pattern. Formaldehyde changes the surface characteristics of type 2 virus considerably but does not similarly alter the surface of types 1 and 3 poliovirus. The surface changes appear before any of the virus is killed. The reasons why type 2 virus can be inactivated more readily than the other two types may be associated with the surface changes noted. The reaction of formaldehyde with the virus protein is therefore not a single-step reaction.

Significance:

The results which have been obtained indicate that polioviruses may be harvested by appropriate methods in a state of purity which would allow them to be crystallized. The separation of virus into nucleic acid and protein indicates that it would be possible to obtain antigenic material which is not viable simply because the protein and nucleic acids exist as separate entities rather than a

combination. With labeled virus and the appropriate techniques it should be feasible to harvest from infected cells virus protein which has not yet been combined with nucleic acid and which would be antigenic in the absence of viability.

The results with formaldehyde treatment of poliovirus indicate why there is such a difference between the three types of poliovirus with regard to their inactivation and their subsequent antigenic potency. Without pure preparations of virus, it is impossible to observe precisely how viricidal agents act to render viral particles innocuous.

Proposed course:

It is planned to continue the present experiments along the same lines with a view toward obtaining pure viral protein and testing it in suitable immunologic systems to determine whether or not it is a nonviable, potent antigen capable of inciting the production of neutralizing antibodies. In addition to this more or less practical result, it is hoped that more can be learned about how mammalian viruses proliferate in tissue-culture cells and at which stage viruses become viable through association of the nucleic acid and protein portions.

Serial No. NIAID-179

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ormsbee, R. A. and Melnick, J. L.: Biologic and serologic characteristics of ECHO viruses from West Virginia. J. Immunol. 79: 384-392. Nov. 1957.

Hoyer, B. H., Bolton, E. T., Ormsbee, R. A., LeBouvier, G., Ritter, D. B., and Larson, C. L.: Mammalian viruses and rickettsiae. Their purification and recovery by cellulose anion exchange columns has significant implications. Science 127: 859-863. April 1958.

Bolton, E. T., Hoyer, B. H., and Ritter, D. B.: Stability of ribonucleoprotein particles of Escherichia coli. Microsomal Particles and Protein Synthesis, Wash. Acad. of Sci., Washington, D. C., pp. 18-21. 1958.

Honors and Awards relating to this project:

Dr. B. H. Hoyer

Courtesy appointment as lecturer at Montana State University, Missoula, Montana.

NIAID
Laboratory of
Bacterial Diseases

Estimated Obligations for FY 1959

Total:	$ 90,200
Direct:	88,000
Reimbursement:	2,200

Covering Project Reports
NIAID....200 thru 203

Serial No. 200
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Brucellosis

Principal Investigator: Dr. Norman B. McCullough

Other Investigator: Dr. Grace A. Beal

Cooperating Units: None

Man Years (calendar year 1958)
Total: 4-1/3
Professional: 1-1/3
Other: 3.0

Patient Days (calendar year 1958)
75

Project Description:

Objectives:

The objectives of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organism, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Clinical studies concern diagnostic criteria for the disease in man, especially in borderline cases of chronic illness in which present cultural and serological techniques fail to provide reliable evidence of infection; studies of the pathogenesis of the disease; and the evaluation of current therapeutic regimens.

Part B included

Methods Employed:

The Warburg Technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

In a collaborative study with members of the Veterinary Department of Purdue University, Brucella melitensis (Old World type) was found to produce generalized and prolonged infection in swine indistinguishable from that produced by Brucella suis. Several strains with low urease activity and other characteristics of the Mediterranean type of Br. melitensis were employed. Each strain possessed minor variant characteristics permitting of definitive recognition. All strains emerged from swine without change in any characteristic. In particular it was established that urease activity plays no role in the determination of pathogenicity of Brucella for swine. This is the first recorded study of experimental infection of swine with classical Br. melitensis (paper in press).

In a further collaborative study with members of the Veterinary Department of Purdue University it was established that Brucella neotomae is pathogenic for swine. Infection of moderate degree was produced. The clinical characteristics were not different from those obtainable with Br. suis.

Studies of an organism isolated from a genital disease of sheep in New Zealand and California, and placed in the genus Brucella by the original investigators, have been completed. This organism does not have the characteristics of the present members of the genus, is totally dissimilar antigenically, and should not be considered a member of the genus Brucella.

The difficulties experienced in antigenic typing of Brucella led us to reinvestigate the antigenic relationships in the genus. These studies are not finished, but data to date indicate that the use of monovalent typing sera has confused rather than facilitated typing of Brucella. Our data establish three antigenic groups of Br. melitensis, and clearly distinguish the American type of Br. melitensis from Br. abortus and Br. suis.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) McCullough, N. B.: Human Brucellosis, with Special Reference to the Disease in the United States. Ann. N. Y. Acad. Sc. 70: 541-556, June, 1958.

(2) McCullough, N. B. and Beal, G. A.: The Biological Stability of the Genus Brucella. Bull. Wld. Hlth. Org. 19: 725-737, 1958.

(3) Beal, G. A., Taylor, D. B., McCullough, N. B., Claflin, R. M. and Hutchings, L. M.: Experimental Infection of Swine with Brucella Melitensis (Mediterranean Type). In press - Am. J. Vet. Res.

Honors and Awards relating to this project: None

Serial No. 201
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Basic Studies on Staphylococcus

Principal Investigator: Dr. Norman B. McCullough

Other Investigator: Dr. Elizabeth Verder
Dr. Grace Beal

Cooperating Units: None

Man Years (calendar year 1958)
Total: 1
Professional: 7/12
Other: 5/12

Project Description:

Objectives:

1. To develop an objective means of measuring virulence of Staphylococci.

2. To determine the factors responsible for virulence.

3. To determine the significance of such factors in antibiotic resistant strains and the relationship (if any) between the ability to develop antibiotic resistance and pathogenicity.

4. To study the mechanism of antibiotic resistance in Staphylococcus.

Part B not included.

Methods Employed:

The use of experimental animals and tissue cultures in defining overall virulence, and in study of individual fractions or products of the Staphylococcus.

Use of physical and chemical methods in fraction of the organism and its products to allow assessment of individual factors in pathogenicity and in cell injury.

Nutritional studies on the production of factors concerned in pathogenicity, and in antibiotic resistance.

Enzymatic studies, particularly those employing the Warburg technique, for determining the role of particular enzyme systems in antibiotic resistance and in pathogenicity.

The techniques of immunology, genetics, bacterial transformation, and phage induction in exploring the mechanism of development of antibiotic resistance.

Major Findings:

New project.

Significance to the Program of the Institute:

The Staphylococcus has become an important public health problem due to the rapid development of antibiotic resistant strains and their spread through the population. Control of this situation is dependent on the acquisition of more and extensive basic information on the Staphylococcus and on the mechanism of antibiotic resistance. The problem of antibiotic resistance is not limited to the Staphylococcus but is currently becoming of importance in other groups of organisms. Indeed, the development of resistance to external agents is a fundamental phenomenon in biology and deserving of intensive study. The currently described project is designed to provide basic knowledge in regard to the Staphylococcus in particular, but also concerns the broad problem of induced resistance in general.

Proposed Course of Project:

As described above.

Serial No. 202
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Identification of Pseudomonas

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958)
Total: 8/12
Professional: 3/12
Other: 5/12

Project Description:

Objectives:

a) Identification of strains for other scientists.

b) Orientation of scientists in methods used for study of biochemical characteristics of various species and for serological typing.

c) Distribution of cultures of strains of various species and of representative serological types of Ps. aeruginosa on request to research scientists.

d) Maintenance of a large collection of cultures.

Part B not included.

Methods Employed:

The methods which we have employed are ones developed or modified in the course of our studies on the classification of Pseudomonas; the studies were initiated during work on our project on diarrheal disease of the newborn and have been carried on for eight years. A proposed scheme for the antigenic analysis of strains of Ps. aeruginosa was worked out and has been employed successfully in identifying strains by serological typing.

Major Findings:

Methods have been established for use in the identification of individual strains of Pseudomonas so that epidemiological surveys in relation to the spread of resistant strains may be carried out successfully.

Significance to the Program of the Institute:

Methods have been developed for studies on the spread of a group of antibiotic resistant organisms of medical importance, particularly in infections in individuals with minimal resistance such as newborns, the aged, children with leukemia, individuals with extensive or deep burns, etc.

Proposed Course of Project:

Further work should be pursued when the pressure of current studies lessens or additional assistance is available.

Serial No. 203
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Bacteria Characterized by the Production of Reproductive filterable Granules

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: Dr. Alexis Shelokov was co-investigator on the project at time the organisms were isolated and any reports will be made jointly.

Cooperating Units: None

Man Years (calendar year 1958)
Total: 5/12
Professional: 2/12
Other: 3/12

Project Description:

Objectives:

To learn more about 1) the classification of bacteria capable of producing reproductive filterable granules, 2) the environmental changes that induce the formation of granules, 3) the potentialities of these incomplete filterable organisms in the establishment of infectious processes and 4) the factors influencing the development of more complete cells from filtered particles.

Part B not included.

Methods Employed:

Through the use of special media, organisms of this type have been isolated from the peripheral blood of several patients with illnesses with an indefinite diagnosis. Methods commonly employed in the study of organisms producing L bodies and the pleuro-pneumonia-like (PPLO) group were utilized.

Major Findings:

More detailed studies must be carried out before significant findings may be reported.

Significance to the Program of the Institute:

Organisms with similar characteristics have been recognized as important etiological agents of animal disease for many years. Their presence in extracts of tissue and blood serum collected from infected embryo or animals and used inadvertently in the preparation of tissue culture media has increased interest in the development of more satisfactory methods for their detection and identification. Their importance in infectious processes in man has not been adequately assessed. Since several species of Bacterioides are present in large numbers in the intestinal canal of man and animals, some of these organisms may belong to closely related species possessing filterable granules under certain circumstances and be present in the peripheral blood frequently. The importance of the group to medical scientists is emphasized by the three day conference sponsored by the N. Y. Academy of Sciences to be held in January for discussion of various phases of studies on this group of organisms; Drs. Klieneberger-Nobel and Edwards of London and Dr. Freund of Copenhagen will be among the invited participants.

Proposed Course of Project:

No additional work will be carried on with these organisms until the studies on the staphylococcus have been well established, unless other investigators undertake to contribute to the studies.

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